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POSTPARTUM INFLAMMATION MODULATION AND CALCIUM SUPPLEMENTATION EFFECTS ON COW METABOLISM, HEALTH, AND PERFORMANCE IN DAIRY COW

A Thesis in

Pathobiology

by

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ABSTRACT

The objective of this study was to assess the effects of the combination of postpartum acetylsalicylic acid (ASA) administration and calcium supplementation on haptoglobin (Hp) concentration, calcium (Ca) concentration, beta-hydroxybutyrate (BHB), and the incidence of subclinical ketosis, subclinical hypocalcemia, and clinical metritis in multiparous (≥2 lactation) cows. Within 12 h after calving, cows were randomly allocated to one of four treatment groups: 1) ASA (n=155) = Cows received two oral administrations with ASA 24 h apart (125 g/cow/d; 4 480-grain aspirin boluses); 2) ASACAL (n=164) = Cows received two oral administrations with ASA (125 g/cow/d; 4 480-grain aspirin boluses) and Ca (43 g/cow/d; 2 Ca boluses) 24 h apart, 3) CAL (n=171) = cows received two oral administrations with Ca (43 g/cow/d; 2 Ca boluses) 24 h apart, and 4) UNT (n=156) = cows remained untreated. Blood samples were collected before each treatment and at 7±3 DIM to assess Ca, Hp, and BHB concentrations. Cows that presented a BHB concentration of >1.2 mmol/L were classified as having subclinical ketosis, while a Ca concentration <8 mg/dL was classified as subclinical hypocalcemia. Vaginal discharge was scored weekly from calving until 21±3 DIM for assessment of clinical metritis, defined as the presence of a brownish fetid vaginal discharge. On-farm records were collected to assess health events in the first 60 DIM, DHIA test data, reproductive performance, and culling for 300 DIM. The data were analyzed using MIXED, GLIMMIX, and LIFETEST SAS procedures. There was no difference in BHB or Hp concentrations between treatment groups. Cows that became sick in the first 60 DIM had lower concentrations of Ca and higher concentrations of BHB and Hp in the first week after calving compared to cows that remained healthy. Intriguingly, cows treated with ASA or ASACAL tended (p=0.09) to have lower concentrations of Ca at the second treatment administration compared to UNT (ASA= 7.66±0.07 mg/dL; ASACAL= 7.64±0.06 mg/dL; UNT= 7.90±0.07 mg/dL). Cows treated with CAL tended (p=0.1) to have a lower prevalence of subclinical ketosis compared to UNT cows (CAL=36.96±4.95%; UNT=46.63±5.19%). On the other hand, cows treated with ASA tended (p=0.1) to have a lower prevalence of clinical metritis compared to CAL cows (ASA=15.92±3.56%; UNT=29.92±4.40%). Regarding 4% DHIA fat-corrected milk, there was a test*treatment*parity interaction (*p*=0.03). Second lactation ASACAL cows produced 1.54 kg, 3.4 kg, 1.45 kg, and 1.27 kg less fat-corrected milk in DHIA tests 1, 2, 4, and 5, respectively, compared to second lactation UNT cows. Conversely, third+ lactation ASACAL cows produced 3.42 kg and 3.53 kg more fat-corrected milk in DHIA tests 2 and 3, respectively, compared to third+ lactation UNT cows. Similarly, second lactation ASACAL cows tended to produce 537 kg less ME305 milk compared to second lactation UNT cows, while third+ lactation ASACAL cows tended to produce 443 kg more ME305 milk than third+ lactation UNT cows. ASACAL cows tended to require fewer days (*p*=0.10; ASACAL=101.77±4.18 d; UNT=111.45±4.13 d) and fewer services (*p*=0.05; ASACAL= 1.91±0.13 svc.; UNT=2.27±0.13 svc.) to become pregnant compared to UNT cows. Interestingly, third+ lactation ASAPCAL cows had a lower fat percentage in the DHIA test 1 compared to third+ lactation UNT cows. These findings suggest that postpartum acetylsalicylic acid administration alone or in combination with Ca supplementation may alter Ca metabolism. Furthermore, these results strengthen the existent knowledge regarding the adverse effects of postpartum inflammation and poor Ca and fat tissue metabolism on cow health. In addition, postpartum acetylsalicylic acid combined with Ca may improve milk yield in third+ lactation cows, while it may be detrimental for second-lactation cows. Moreover, ASACAL treatment might positively affect fertility in multiparous cows.

Keywords: Postpartum period, Calcium and acetylsalicylic acid, Cow metabolism and health

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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THESIS DEDICATION

I would like to dedicate this thesis to my family, Mohammad Sadegh Zarei, Zohreh Momeni and Pouria Zarei. I am deeply appreciative of the sacrifices they made to provide me with opportunities and the constant encouragement they provided to pursue my dreams.

.Chapter 1

Introduction

Transition period referred as 3 weeks prior to 3 weeks after calving is a critical period in dairy cattle life cycle in terms of health, production, and reproductive performance. During this period, dairy cows experience several physiological challenges such as decreased dry matter intake (DMI), negative energy balance (NEB), body fat mobilization, systemic inflammation (SI), and impaired immune system. Altogether, these challenges predispose cow to increase chances of developing metabolic and infectious diseases including subclinical ketosis, hypocalcemia, and clinical metritis. To help periparturient cows pass more efficiently through this period, several management approaches have been proposed. Among those practices, use of anti-inflammatory treatments such as nonsteroidal anti-inflammatory drugs (NSAIDs) and oral calcium (Ca) supplementation have been the point of interest and have widely been researched and investigated in recent years. In this regard, Acetylsalicylic Acid (ASA) commonly known as Aspirin has been suggested by literature as one of the NSAIDs to modulate post-partum SI. However, the probable synergistic effects of a combined treatment protocol including ASA administration and Ca supplementation have never been studied before. Therefore, the objective of this study was to assess the effects of the combination of ASA administration and Ca supplementation after calving on metabolism, health, milk yield, milk components, reproductive performance, and culling in multiparous Holstein dairy cows. We hypothesized that this treatment approach would boost health and performance in post-partum multiparous dairy cows.

Chapter 2

Literature Review

1. The Transition Period

The transition period, spanning the three weeks before and after calving, is a critical phase in dairy cattle's production cycle, which significantly challenges the health, productivity, and profitability of dairy herds (Drackley, 1999). During this time, cows undergo dramatic physiological changes as they transition from a non-lactating late pregnancy stage to an early lactation non-pregnant stage, predisposing them to an increased risk of metabolic and infectious diseases. Some of these changes, which negatively affect metabolic and immune responses, are closely associated with developing many transition cow disorders (Contreras et al., 2010; Sordillo et al., 2009). This period is characterized by a delicate interplay of metabolic, hormonal, and immunological adjustments, marking the initiation of milk production and the adaptation of the cow's body to the demanding metabolic requirements of lactation. Therefore, successful management of the transition period is of high importance for ensuring optimal milk production, reproductive performance, and overall herd welfare.

As one of the most notable physiological changes, excessive mobilization of body fat stores and subsequent increases in plasma fatty acid concentrations are considered as important risk factors for postpartum diseases (Dechow et al., 2004). While feed intake is maintained at normal levels during non-lactating periods, it starts dropping sharply around one weeks prior to parturition (Gross et al., 2011). This drop in dry matter intake (DMI) could remain until around one week after calving (Grummer et al., 2004). A study showed that although decreased intakes of dry matter occurred on the calving day in cows from all different parities, the percentage of drop varies from around 25% in the cows in their first or second lactations to 52% in the cows with third lactation and greater with more extreme percentages being observed in the paretic cows and the ones experiencing milk fever (Marquardt et al., 1977). As the cow gets closer to parturition, and after the parturition, cows experience an increase in energy and nutrient demands required for fetal development and milk yield respectively. This along with the drop in DMI and fat tissue mobilization will predispose cow to enter in a negative energy balance (Ingvartsen, 2006; Zhang et al., 2020). This status could be exacerbated by common factors that affect dairy cows such as heat stress or other management issues, which further reduces DMI (Rhoads et al., 2009; West, 2003). During times of NEB, body energy resources, such as the fat tissue, must be mobilized to support energy-dependent needs in the body. A wide set of dramatic adaptive challenges take place as lactogenesis, uterine involution, and the accompanying changes in endocrine and metabolic states happen at the same time (Bradford et al., 2015).

2. Systemic Inflammation During the Transition Period

Inflammation is a naturally occurring response to different sorts of stimuli and counts for alleviating infection and tissue injuries. According to previous research, a regulated postpartum inflammatory response is critical for a gradual adaptation to a new physiological state. This includes the involution of mammary gland (Atabai et al., 2007), remodeling of adipose tissue (Kosteli et al., 2010), and expulsion of the placenta (Challis et al., 2009). Although controlled inflammatory response is considered beneficial as it can provide an initial protection against the stimuli, it can turn into a detrimental state if dysregulated and exacerbated (Medzhitov, 2008).

At its onset, an acute inflammatory response, which can be triggered by infection or tissue injury, causes a coordinated production and delivery of blood metabolites and immune cells to the infection or injury site (Kumar et al., 2017; Majno & Joris, 2004). As the acute inflammatory stimulus progresses, expression and release of inflammatory metabolites including cytokines, chemokines, adhesion molecules, eicosanoids, and complement proteins increase substantially (Newton & Dixit, 2012). Aggregated activity of these molecules will form complex and complicated regulatory networks to promote increased blood flow to infected tissue, immune cells activation and infiltration, and systemic responses, including increased body temperature, increased heart rate, and decreased appetite (Dantzer & Kelley, 2007). It has been shown that different cytokines such as tumor necrosis factor α (TNFα), IL-1β, and IL-6 which are produced by many cell types, especially macrophages and mast cells could play important roles in the inflammatory response by activating leukocytes and endothelial cells as well as triggering the acute-phase response (Bannerman et al., 2009; Bradford et al., 2015). Horst et al. (2021) categorized the transition inflammation into different types based on the source of the inflammation. They argued the peri-parturient inflammatory response could be associated with a broad variety of immunogenic and pathogenic components at three prominent sources in the transition cow which are the uterus, mammary gland, and gastrointestinal tract.

Based on the literature, those cows who are unable to successfully adapt to the peripartum challenges are more susceptible to an impaired immune system and an exacerbated and prolonged inflammatory response during the postpartum period (Pascottini et al., 2022). This exacerbated inflammation is associated with an increase in blood concentrations of positive acute-phase proteins (i.e., haptoglobin), and pro-inflammatory cytokines, such as IL-1β, IL-6, and TNFα (Trevisi & Minuti, 2018). These positive acute-phase mediators are shown to be elevated in the days after parturition with or without any signs of disease or disorder (Akbar et al., 2015; Bionaz et al., 2007; Graugnard et al., 2012; Huzzey et al., 2009; Mullins et al., 2012). For instance, it has been shown that while a higher increase of inflammatory markers is observed in the cows experiencing diseases or calving difficulties, serum haptoglobin concentration is elevated in around parturition in healthy cows (Qu et al., 2014).

3. Anti-inflammatory management in transition period

Several studies have experimented with use of non-steroidal-anti-inflammatory drugs (NSAIDs) during the transition period in order to control exacerbated inflammation, as well as pain and discomfort, associated with the calving process. A subclass of inflammatory mediators known as eicosanoid group is of particular relevance in regard to the mechanism of action of NSAIDs. These mediators are formed from the phospholipids located in the cell membrane in response to inflammatory damages (Lees et al., 2004). As the signs of inflammation begin, a polyunsaturated fatty acid (PUFA) named arachidonic acid is released from the cell membrane phospholipids under a series of regulations. Arachidonic acid can serve as a substrate for different groups of enzymes, among which Cyclooxygenases (COXs) are of high importance. Through a series of cascade reactions, involving the actions of further enzymes, different groups of eicosanoid mediators including prostaglandins (PG) and thromboxane A_2 (TxA_2) are formed from COX activity. These mediators subserve several roles during the inflammation process. However, as they are predominantly activated before the actual inflammatory reactions start, they are usually referred to as pro-inflammatory mediators (Lees et al., 2004).

It has been reported that NSAIDs have anti-inflammatory, analgesic, and antipyretic effects, as well as platelet aggregation inhibition effects (Gøtzsche, 2010). Non-steroidal-anti-inflammatory drugs inhibit cyclooxygenase enzymes (COX; prostaglandin synthase) as their primary effect results in altering the ultimate transformation of arachidonic acid to eicosanoids such as prostaglandins, prostacyclin, and thromboxanes (Lees et al., 2004). These cytokines are initially produced by COX during the inflammation. There are two related isoforms of the COX enzyme in the body. The first isoform is COX-1(PGHS-1), which is expressed in most of the tissues and is stimulated either by hormones or growth factors. It is associated with the regulation of physiological cellular processes (Trimboli et al., 2020). On the other hand, the second isoform is COX-2 (PGHS-2), which has been shown to be expressed mainly in the brain, kidney, bone, and probably in the female reproductive system (Trimboli et al., 2020). The COX-2 enzyme is initially activated under the transcription and expression of a gene that is rapidly inducible and tightly regulated. Under normal conditions, COX-2 expression is highly restricted, but it is dramatically upregulated during inflammation. Most of the known side effects of NSAIDs such as gastrointestinal irritation, renal toxicity, and inhibition of blood clotting are mostly related to the use of the second group (Lees et al., 2004). Several studies have been conducted on understanding the effects of different types of NSAIDs on dairy cattle.

3.1 *Non-steroidal-anti-inflammatory drugs*

3.1.1 *Flunixin Meglumine*

The N-methyl-d-glucamine salt of flunixin (flunixin meglumine, FM) is an NSAID with terminal half-life from 3.14 to 8.12 hours after gradual IV administration. This NSAID inhibits both cyclooxygenase isoforms COX-1 and COX-2, but is more selective for COX-1 (Anderson et al., 1990; Lees et al., 2004). Flunixin meglumine is labeled for treatment of pyrexia, modulation of inflammation caused by endotoxemia, and other complications such as bovine respiratory disease and acute bovine mastitis (FDA, 2004). The first study on FM was conducted by Waelchli et al. (1999) 24 years ago to investigate the effects of FM on the risk of retention of fetal membranes after a caesarean section on a total of 98 cows. The results showed that cows treated with FM were three times more susceptible to developing retained placenta compared to the control group. Shwartz et al. (2009) tested FM administration within 3 DIM on 26 multiparous cows and found that FM slightly increased rectal temperature, reduced overall DMI and plasma urea nitrogen, and did not have any considerable effects on milk yield, milk fat, overall energy balance, NEFA and plasma glucose. Similarly. Newby et al. (2017) investigated the effects of two different protocols for FM treatment on both prepartum and postpartum Holstein cows. They suggested that treatment with FM is not recommended due to severe negative side effects such as high rate of stillbirth, retained placenta, increased risk of fever, increased risk of developing metritis and decreased milk yield.

More recently, Schmitt et al. (2023a, 2023b) investigated a single transdermal administration of FM on milk yield, culling risk, reproductive performance, inflammatory and metabolic markers, uterine health, and indicators of pain in postpartum Holstein Friesian dairy cows. A total of 500 cows including 153 primiparous and 347 multiparous were treated with a single dose of FM between 24 to 36 h postpartum. They found that primiparous cows treated with FM showed a slight reduction in inflammation, represented by lower HP, and decreased risk of metritis along with increased milk, milk fat, and milk protein yields. Moreover, multiparous cows treated with FM showed improved metabolic profile specifically highlighted by reducing BHB compared to the control group but had decreased milk parameters.

3.1.2 *Carprofen*

Carprofen (CP) [(±)-6-chloro-α-methylcarbazole-2-acetic acid] is a powerful NSAID, which has a half-life of 44 to 64 hours (Miciletta et al., 2014). It has higher selectivity for the COX-2 isoform compared to FM. Meier et al. (2014) studied the effects of CP on approximately 640 early or late postpartum pasture-grazed dairy cows. Cows were treated with 1.4 mg/kg of CP and were analyzed to investigate the effects of CP on milk production, metabolic status, uterine health, and reproductive performance. To determine the right timing of CP administering, two different protocols were selected. In the first protocol, CP was administered on day 1, 3, and 5 postpartum while in the second protocol it was administered on days 19, 21 and 23 after calving. The results illustrate that irrespective of timing of administration, there was not any significant changes on milk yield, body weight, or body condition scores during early lactation. Moreover, no detectable effect was observed regarding the effects of CP on pregnancy rates, uterine health or circulating metabolites and minerals. The only notable difference in treatment groups was that early CP treatment group had marginally lower serum β-hydroxybutyrate concentrations.

Stilwell et al. (2014) treated 19 cows with an CP IV injection utilizing a dose of 1.4 mg/kg within 6 hours after calving. These results suggest that there was no difference in clinical disease incidences over the 3 days period postpartum. However, more animals from the CP group were observed eating during the first hours after calving. Additionally, the rectal temperature was lower on CP treated cows compared to placebo cows. However, fewer cows were pregnant by 220 DIM in the treated group.

Giammarco et al. (2018) evaluated the effects of a single postpartum injection of FM or CP on hematological indicators, productive performance, and fertility. The first group received intramuscular FM (2.2 mg/kg), the second group received subcutaneous CP (1.4 mg/kg) and the third group received subcutaneous saline as a control at 2.0 mL/45.5 kg of BW within 12 hours after calving. The results showed that there were no significant effects on rectal temperature, heart rate, overall milk yield, milk composition, dry matter intake, BW and body condition score, serum metabolite concentrations of glucose, non-esterified fatty acids, β-hydroxybutyrate, serum urea nitrogen, total protein, and albumin. However, FM-treated multiparous cows had a significantly lower prevalence of retained placenta compared to both control and CP-treated cows. Furthermore, a greater percentage of cows became pregnant at the first insemination in both NSAIDs groups compared to the control group.

Most recently, Rodríguez et al. (2021) evaluated the effects of early treatment with CP on clinical metritis (CM) incidence and reproductive performance in 62 multiparous cows at the beginning of the close-up period. Subcutaneous CP administration (1.4 mg/kg) were implemented within the first 12 to 48 hours after calving. Carprofen treatment decreased prevalence and risk of developing CM by 29.5 percent points and 3.4 times respectively. Also, CP administration decreased by 9 days the chances to become to become pregnant for 50% of cows at first service after day 60 by 9 days but did not have any effects on other reproductive performance parameters.

3.1.3 ***Meloxicam***

Meloxicam (MEL), an enol-carboxamide NSAID related to piroxicam, has been proposed to treat acute inflammation and pain for a long time (Bekker et al., 2018). Contrary to other NSAIDs, its inhibitory activity against COX-2 is greater than COX-1 (Engelhardt, 1996). Meloxicam is predominantly a COX-2 inhibitor with high oral bioavailability and half-life of approximately 26 hours in cattle (Malreddy et al., 2013). Although when administered via injection at 0.5 mg/kg of body weight (BW), the milk withdrawal time of MEL is 5 days, oral use of MEL at 1 mg/kg of BW has undetectable residues in milk after 80 h.

Newby et al. (2013) performed an experiment where they enrolled 103 dairy cows and treated them with a subcutaneous injection of MEL (0.5mg/kg) 24 hours after calving, reporting no effects of treatment on DMI, milk production, blood metabolites, or health events. A year later, Newby et al. (2014) investigated the effects of MEL on the risk of retained fetal membranes with same treatment protocol and no differences were observed between treatment groups. Similarly, Mainau et al. (2014) assessed the effects of MEL on various physiological and behavioral measures, which has been associated with pain in cattle. Within an average of 3.4 hour after calving, 60 dairy cows (primiparous and multiparous) received a subcutaneous MEL administration (0.5mg/kg). The results showed that while the post-calving activity was significantly increased in meloxicam-treated heifers, no significant effects on milk production or acute phase responses proteins were observed compared to untreated cows.

Carpenter et al. (2016) implemented a study aimed at evaluating the effects of sodium salicylate (SAL) and MEL during the postpartum period. In the study, 51 multiparous dairy cows received either oral MEL treatment (675 mg/cow) or oral sodium salicylate administration (125mg/cow) within 12 to 36 hours after parturition. The authors reported that whole lactation milk and protein yields were greater in both NSAID-treated cows compared to the control. Furthermore, MEL treatment increased concentrations of blood glucose compared to both SAL and control group in older cows.

Shock et al. (2018) assessed the effects of administration of a single dose of oral MEL on the production and health status in a study where 2,653 cows were enrolled. The results indicated that MEL treated cows produced 0.64 kg/day more milk on the first 3 DHIA tests, had 0.75 times the risk of developing subclinical mastitis in the first test, and 0.46 the risk of be culled or died before 60 days in milk.

Swartz et al. (2018) tested effects of oral MEL administration (1mg/kg of BW) on the behavior, health, and production of peripartum cows, both pre- [MEL-PRE] and post- (MEL-POST) calving. Although there was no effect of treatment on health outcomes, eutocic MEL-PRE animals produced 6.8 kg/d more milk compared to eutocic control group within the first 15 weeks of lactation. Similarly, MEL-PRE animals produced more milk fat, protein, and lactose (kg/d) than the control group.

Lastly, Pascottini et al. (2020a) evaluated the effects of MEL on markers of systemic inflammation and energy metabolism, as well as endometritis incidence, in clinically healthy postpartum dairy cows. In this study, 20 cows received MEL (0.5 mg/kg of BW) once daily for 4 days (10–13 days postpartum). Authors reported that MEL reduced haptoglobin concentration, and improved indicators of energy metabolism such as lower BHB concentrations.

3.1.4 ***Acetylsalicylic acid***

Several studies have been conducted to investigate effects of Acetylsalicylic acid (ASA) administration before or after calving. Barragan et al. (2020a) aimed to assess effects of postpartum ASA administration on different health biomarkers of cows under certified organic management. Based on their results, not only did cows treated with ASA produce 1.82 kg/d more milk than cows treated with placebo (PLC) during the first 30 DIM but dystotic cows that received ASA produced 4.48 kg/d more milk compared to the PLC dystotic cows. In addition to that, ASA treated cows tended to require fewer days and fewer services to become pregnant compared to PLC cows. Therefore, they concluded that at least under certified organic management, treating post-partum cows with ASA may improve milk production and udder health, as well as increasing activity and enhancing fertility.

In the same year, Barragan et al. (2020b) performed a similar study to further assess effects of ASA administration targeting inflammation and stress status. With a smaller number of cows, they reported that second+ lactation (MULT) cows that were treated with ASA had lower concentration of Hp compared to PLC cows and concluded that a short duration of post-partum ASA administration reduces Hp in MULT cows without having any effects on Substance P (SP) and cortisol concentrations.

In another study from Barragan et al. (2021), less number of the post-partum cows that received ASA were diagnosed with clinical metritis at 7±3 DIM and clinical endometritis at 50 ± 10 DIM compared to the untreated cows. Additionally, they reported that ASA treated cows had a better reproductive performance compared to untreated cows. Based on these findings, ASA administration after calving was shown to have the potential to decrease the incidence of uterine diseases and improve reproductive performance.

4. Important Metabolic Diseases During the Transition Period

4.1.1 Ketosis

Ketosis is one of the most common metabolic diseases in early lactating dairy cows characterized by high concentrations of circulating ketone bodies, which can lead to productive and reproductive losses, and even death or early culling (McArt et al., 2015; Mostert et al., 2018). There have been multiple studies regarding the incidence and economic impacts of ketosis. In a study, the average prevalence of subclinical ketosis (blood BHB threshold $\geq$1.2 mmol/L) within ten European countries was shown to be 21.8% (ranging between 11.2 and 36.6%) between the 2nd and 15th day of lactation (Suthar et al., 2013). In another study (Overton et al., 2017), an overall incidence (total cases per day) of hyperketonemia was estimated to be greater than 20%, corresponding to an overall incidence (new cases in cows at risk per day) of 40%. Another study showed that the prevalence of subclinical ketosis was around 17% in South Africa, 13.3% in Central and South America, 28.5% in Asia, and 24.85% in Oceania (Brunner et al., 2019). With taking a closer look at all these numbers, it is obvious that irrespective of varying frequency in different countries, hyperketonemia stands as a major issue on dairy farms.

At the onset of lactation, milk production demands a 30 to 50% increase in energy for the cow (Gruber & Mansfeld, 2019). This critical need for higher energy for milk production is even more exacerbated in high producing cows. These requirements are reflected by the plasma glucose concentrations that decreases after calving. Glucose is required as a precursor for synthesis of lactose in the mammary gland, which is the main sugar (disaccharide carbohydrate) in the milk. Lactose also is the main osmotic regulator for the mammary secretion of water, determining the total volume of milk produced (Lei & Simões, 2021). Thus, around 85% of the glucose produced at the beginning of lactation is destined for the mammary gland (Knegsel et al., 2014). Also, integral roles of glucose as an essential nutrient associated with the maintenance of the normal vital functions has been shown. However, due to the substantial drop in DMI around delivery, this contribution is not enough to meet requirements. As stated earlier, all these factors together culminate in the occurrence of NEB. Because of the fact that available quantities of glucose obtained by gluconeogenesis are insufficient in periods of NEB, the body needs to urgently mobilize alternative energy sources as a compensation. The first and foremost source of energy is the fat reserves stored in the adipocytes in the fat tissue (Zhang & Ametaj, 2020). These fat reserves are usually stored in the form of triglyceride (TG). The lipolysis process of TG in adipocytes, results in production of glycerol and non-esterified-fatty-acids (NEFAs) that are released into circulation and sent to the liver. The presence of high concentrations of these circulating fatty acids negatively affects the insulin signaling pathways, leading to a decreased sensitivity (Gao et al., 2018). Most studies on this topic have confirmed that dairy cows go through an insulin resistance period between the end of pregnancy and the beginning of lactation, which seems to be a physiological, adaptive, and transitory mechanism that aims to prioritize the supply of glucose to the pregnant uterus and the mammary gland over other tissues (Koster & Opsomer, 2013). This insulin resistance which is associated with higher concentrations of circulating fatty acids exacerbates the mobilization of fat reserves and the entry into circulation of NEFA and creates a vicious cycle (De Koster et al., 2015; Lei & Simões, 2021).

Upon transferring to the liver, glycerol is used for gluconeogenesis which is a metabolic pathway that results in the biosynthesis of glucose from certain non-carbohydrate carbon substrates. On the other hand, NEFA are converted to acetyl-CoA and can have various destinations. Some of the possible destinations are included but not limited to oxidation to carbon dioxide, oxidation to ketone bodies, hepatic storage in the form of TG, or incorporation into very-low-density lipoproteins (VLDL), which will be exported as fuel for other tissues (Lei & Simões, 2021). In certain timepoints and demanding phases, such as the beginning of lactation, the amount of fat reserves that are mobilized is much higher than normal, leading to the production of a large amount of acetyl-CoA. If the Krebs cycle is not able to fully metabolize the excessive acetyl-CoA, this is transformed into ketone bodies such as acetone, acetoacetate, and β-hydroxybutyrate

(BHB) by different pathways including ketogenic, dehydrogenization and decarboxylation (Furken et al., 2015; White, 2015). As a result, an increased amount of ketone bodies enters the circulation and could also appear in milk and urine in short time (Ingvartsen, 2006).

3.1.2 *Ketosis classification*

Ketosis can be classified according to different parameters. Currently, there are mainly two approaches of classification widely accepted in the literature. Firstly, it could be classified based on etiology and timing (Herdt, 2000; Oetzel, 2015). Based on literature, it is divided into primary and secondary ketosis. Meanwhile, another type which is the ketosis associated with butyric acid silage has been proposed by literature.

Type I ketosis, also known as primary or described as spontaneous ketosis, is referred to the hyperketonemia that occurs in the period between 3 and 6 weeks postpartum. It is referred to as type I ketosis because it is similar to type I diabetes mellitus in humans (G. Zhang & Ametaj, 2020). The time that type I ketosis occurs concord with the peak of lactation when the necessary glucose for milk production exceeds the amount of available glucose. At this phase, glucose precursors that should mainly be provided from the diet (mostly propionate) or muscle protein (in the form of amino acids) are insufficient and will result in a state of chronic hypoglycemia, triggering hypoinsulinemia (Lei & Simões, 2021). In response to these changes, lipolysis and ketogenesis pathways are activated and fatty acids and ketone bodies are used to compensate glucose deficiency and meet energy requirements (G. Zhang & Ametaj, 2020).

On the other hand, type II or secondary ketosis occurs at the onset of lactation right after the parturition due to the intensive mobilization of adipose tissue. This form of ketosis is also named as type II ketosis because of the similarity to human type II diabetes mellitus (G. Zhang & Ametaj, 2020). This intensive mobilization of fat results in the production of high amounts of NEFA. If the gluconeogenesis and ketogenesis pathways are not activated as vigorous as required, the excessive amounts of NEFA are re-esterified in TG. Ruminants generally have limited ability to produce low-density lipoproteins (LDL) to export TG to other tissues. Given this fact, a large part of the TG produced by re-esterification of NEFA accumulates in the liver, resulting in hepatic steatosis (Lei & Simões, 2021). Also, this high concentration of ketone bodies causes an insulin resistance state which results in impairing the use of glucose. Higher concentrations of both blood insulin and glucose are observed in cows diagnosed with type II ketosis (Holtenius, 1996). Higher body condition scores (BCS) and overfeeding during the dry period are considered as two critical risk factors for type II ketosis (Lei & Simões, 2021).

The third type of ketosis that has been proposed by literature is associated with the consumption of silage enriched with butyric acid. This type of ketosis happens when the cow is fed with rations that consist of high concentrations of ketogenic precursors such as butyric acid (Tveit et al., 1992). Although it is preferred for the carbohydrates to be fermented into lactic acid in the silage, sometimes those are fermented into butyric acid instead (Zhang & Ametaj, 2020). Cows show signs of butyric acid silage ketosis when they ingest large amounts of silages that have undergone *Clostridial* fermentation. However, it is not the only factor that leads to this type of ketosis, depending on the presence of other risk factors, including ruminal acidosis, high milk production, low dietary energy, and high dietary protein (Oetzel, 2015).

The second general classification of ketosis is based on the concentrations of BHB in the blood and the lack or presence of clinical signs of disease. Based on that, ketosis is divided into two forms including subclinical ketosis (SCK) and clinical ketosis (CK). Subclinical ketosis is referred to as an increase in the concentrations of ketone bodies in the blood, urine, or milk, when there are no obvious clinical signs of disease. Cows are keeping their appetite and there is not any considerable decrease in DMI (G. Zhang & Ametaj, 2020). According to literature, concentration of BHB in the serum at the times of SCK is between 1200 to 1400 μmol/L (1.2 to 1.4 mmol/L) and it is generally used as a means for diagnosis of SCK (Brunner et al., 2019; Suthar et al., 2013). Clinical ketosis on the other hand is characterized by the simultaneous occurrence of hyperketonemia along with the presence of clinical signs including lower appetite, loss of body weight, decreased milk production, and dry manure followed by a hypoglycemia (Gordon et al., 2013). This type of ketosis is usually recognized with blood concentrations of BHB higher than 3000 μmol/L (3 mmol/L; Oetzel, 2015).

4.2.1. ***Hypocalcemia***

Hypocalcemia, also known as milk fever in its clinical presentation, is among the most common post-partum metabolic disorders in dairy cattle. Standing as one of the most economically challenging complications, it has always been a point of significant concern in dairy cattle industry and has perpetually been capturing the attention for more research in order to improve preventative management practices. Hypocalcemia is characterized by a sudden and drastic decrease in the serum concentration of calcium levels after calving, following the onset of lactation and colostrum production. In other terms, the transition from late gestation to early lactation imposes a remarkable physiological demand for calcium, and failure to meet this demand can lead to the onset of hypocalcemia. Although it had commonly been known as milk fever for a long time, later research suggested that the more appropriate term to address the issue could be periparturient paresis, since an elevated body temperature is not necessarily observed (Goff, 2008). Reduced feed intake, decreased rumen and intestinal mobility, lower production, and an increased risk of other metabolic and infectious diseases are some of the results of disturbances in blood calcium concentration (Goff, 2008). It is important to recognize that both clinical and subclinical hypocalcemia not only can significantly lower the likelihood of milk production and fertility of the herd, but they are also considered as a gateway factor that predispose cows to a wide range of other metabolic and infectious diseases (e.g., clinical metritis, ketosis). For instance, the decreased rumen and abomasal motility caused by hypocalcemia raises the possibility of displacement of abomasum. As hypocalcemia decrease feed intake, cows experience a higher mobilization of body fat in early lactation. The risk of mastitis also is increased, caused by a decrease in the contraction ability of all muscles, including the teat sphincter muscle (Goff, 2008).

According to Reinhardt et al. (2011), low concentrations of blood calcium could be a risk factor for post-partum diseases. It also negatively affects reproductive performance in later stages (Caixeta et al., 2017; Martinez et al., 2012). Furthermore, based on the timing and duration, it might negatively affect milk yield (McArt & Neves, 2020; Rajala-Schultz et al., 1999). While only approximately 5% of cows in the U.S. might be affected by clinical hypocalcemia (CH), almost 50% of periparturient cows may experience subclinical hypocalcemia (SCH; Horst et al., 2006). This prevalence rate was repeatedly shown in other studies using diagnostic serum or plasma total Ca thresholds ranging from 2.0 to 2.15 mmol/L at 24 and 48 h postpartum (Martinez et al., 2012; Reinhardt et al., 2011). Considering the relatively low percentage of CH compared to SCH, it is evident that negative effects of the latter is much greater in terms of herd level losses. Along with these studies, there are others that have provided evidences, which shows cows with SCH have 3 to 5 times the likelihood of developing postpartum disease and 50% more likely to be removed from the herd in early lactation compared to the cows with normal Ca levels (Chapinal et al., 2011; Rodríguez et al., 2017; Venjakob et al., 2018). In terms of reproductive losses, cows with serum total Ca concentrations ≤2.15 mmol/L at 1, 2, and 3 days in milk (DIM), had 70% decreased odds of pregnancy to first service compared to normocalcemic cows (Caixeta et al., 2017).

3.2.2 *Ca homeostasis*

It has been shown that Ca plays a number of vital roles in a variety of biological functions such as bone metabolism and mineralization, nerve conduction, muscle contraction, cardiac action potentials, intracellular signaling, blood coagulation, membrane permeability, enzyme activity and hormone release in response to a variety of autocrine, paracrine, and hormonal factors and thus, must be strictly regulated and controlled in both intracellular and extracellular fluids (Brown et al., 1991; Hernández-Castellano et al., 2020; Wilkens et al., 2020). Given the importance of the roles that Ca plays in the body, Ca homeostasis is considered crucial to ensure all the functions and reactions dependent on Ca are performed optimally. Blood calcium concentrations are maintained by intestinal calcium absorption regulation, renal calcium reabsorption and bone calcium resorption (Hernández-Castellano et al., 2020). Calcium homeostasis under normal physiological conditions is mainly regulated by the action of three classic calciotropic hormones: parathyroid hormone (**PTH**), calcitriol and calcitonin (**CT**). These hormones regulate Ca homeostasis in mammals by coordinating the availability of Ca in blood, through the regulation of Ca metabolism in bones, intestine and kidneys (Brommage & DeLuca, 1985; Hoenderop et al., 2005). When blood calcium concentrations decrease, calcium-sensing receptors located on the parathyroid chief cells are activated and PTH is released into the bloodstream (Kumar & Thompson, 2011). Upon release of PTH, this hormone sends signals to the osteoblast membrane and induces osteoclast proliferation which ultimately increases bone resorption activity (Hernández-Castellano et al., 2020). Osteocytes appear to contribute to calcium transported from bones through a process known as osteocytic osteolysis in addition to osteoclastic bone resorption (Wysolmerski, 2012). Apart from elevating bone resorption, PTH additionally prompts the release of the renal 1-α-hydroxylase, an enzyme responsible for converting calcidiol into calcitriol. Other endocrine factors like prolactin, estradiol, and placental lactogen can also stimulate the activity of 1-α-hydroxylase during pregnancy and lactation (Kovacs & Kronenberg, 1997). This process is leading to an increase in calcitriol concentrations as well. As mentioned before, calcitriol is the second hormone that plays a role in regulating Ca concentrations. This hormone which is also known as the active form of vitamin D ($1,25\text{-}(OH)_2D_3$), increases calcium concentrations in blood by increasing both intestinal calcium absorption and renal calcium reabsorption (Horst et al., 1994). Calcitriol intestinal Ca regulation occurs by affecting the calcium absorption mainly through the active transcellular pathway, which consists of calcium entry through specific calcium channels (Lieben et al., 2011).

On the other hand, Ca levels could also increase in response to many different physiological disturbances. Any increase in either cytoplasmic or extracellular Ca concentrations above normal physiological levels (hypercalcemia) would cause CT release into the bloodstream (Garrett et al., 1995). The mechanism of action of CT is primarily opposite to PTH and by targeting the osteoclasts, it will reduce calcium resorption from bones. As described above, apart from these three hormones, there are other endocrine factors that play a role in the Ca regulation process. These factors are included but not limited to prolactin and serotonin, which regulate Ca concentrations through different pathways (Hernández-Castellano et al., 2020).

3.2.3 *Clinical hypocalcemia etiology*

The etiology of hypocalcemia in dairy cattle is directly linked to the abrupt shift in calcium homeostasis during the periparturient period. Normal blood Ca concentrations is maintained between 2.1 and 2.5 mmol/L in adult cows (8.5 and 10 mg/dL; Goff, 2008). As previous research indicates, the nadir in blood Ca concentration reveals within the first 12 to 24 hours after calving (Goff, 2008), and blood Ca concentration decreases during the first 2 to 3 days around calving (Quiroz-Rocha et al., 2009).

Clinical hypocalcemia, also known as milk fever, takes place within 72 hours after parturition, with more than 75% of cases occurring in the first 24 hours post-partum

(Oetzel, 1988; Radostits et al., 1994). Milk fever is divided into 3 stages according to severity and clinical signs. These clinical signs include increased heart rate, weakness, cold ears, muscle tremors, dilated pupils, low rectal temperature, and decreased rumen contractions in a standing (stage 1) or sternal (stage 2) or lateral (stage 3) recumbent position (Couto Serrenho et al., 2021; Kelton et al., 1998). The significant and sudden drop in blood Ca concentration leads to compromised activities of neuromuscular and circulatory related organs and functions and depression of consciousness, which seems to be the initial reason responsible for the clinical signs (Oetzel, 1988). The clinical signs of CH are well defined in the literature but the threshold for the concentration of total blood calcium (**tCa**) has not been consistent. For instance, DeGaris & Lean (2008) defined total blood Ca < 1.4 mmol/L as CH. However, Venjakob et al, (2017) defined CH only for recumbent cows with a serum calcium concentration below 2.0 mmol/L. One year later, Venjakob et al, (2018) defined CH as serum calcium concentration < 2.0 mmol/L in combination with clinical signs. In the later study, authors considered to include the cows who are showing clinical signs but may have not become recumbent yet. It has been shown that the odds of CH increases with age and milk production (Erb et al., 1985; Oetzel, 1988). According to DeGaris and Lean (2008), it is estimated that the risk of CH raises approximately 9% with each subsequent lactation. The incidence risk of CH related to the number of lactation ranges from <1% in primiparous cows to 4 to 10% in multiparous cows (Caixeta et al., 2015; Reinhardt et al., 2011; Venjakob et al., 2017).

3.2.4 *Subclinical hypocalcemia etiology*

Subclinical hypocalcemia has been defined as blood Ca concentrations below a specific threshold without visible clinical signs. Different studies have used different thresholds for defining SCH but irrespective of this threshold, it is important to understand that cows suffering SCH are not showing any clinical signs. Therefore, measuring blood Ca concentration is considered necessary for diagnosis of SCH (Couto Serrenho et al., 2021). There has been a wide range of studies on SCH due to the importance of this metabolic disease. While in older literature, SCH has been classified as serum or plasma total Ca concentration of ≤7.8 mg/dL (≤1.95 mmol/L; Massey et al., 1993), ≤7.5 mg/dL (≤1.87 mmol/L; Goff et al., 1996), or ≤8.0 mg/dL (<2.0 mmol/L; Oetzel., 1988, 1996), more recent studies have used different thresholds. Reinhardt et al. (2011) determined the prevalence of subclinical hypocalcemia in the U.S. dairy herds. They defined SCH as calcium levels <2 mmol/L. This threshold was based on the normal range of calcium that was proposed by Merck Veterinary Manual reference at the time, which was 2.1 – 2.8 mmol/L. A year later, Martinez et al. (2012) defined SCH as a serum Ca concentration ≤8.59 mg/dL (≤2.14 mmol/L) in at least 1 day in the first 3 DIM. Caixeta et al. (2015) defined SCH as concentrations between 6 and 8 mg/dL of total serum calcium (≤ 2 ≥ mmol/L); while Miltenburg et al. (2016) and Rodríguez et al. (2017) defined SCH as serum Ca concentration ≤ 2.14 mmol/L based on the Martinez et al. (2012) study. Venjakob et al. (2017) characterized SCH as cows not affected clinically but with a serum calcium concentration below 2.0 mmol/L.. Caixeta et al. (2017) changed their method of definition compared to their 2015 study and this time, decided to define SCH as serum Ca concentration ≤2.14 mmol/L based on (Martinez et al., 2012).

In the most recent and most precise definition and categorization, McArt & Neves. (2020) defined three different groups of SCH. The first group is characterized as transient SCH (tSCH), which has different thresholds for primiparous and multiparous cow. For primiparous cows, the threshold was Ca ≤2.15 at 1 day after calving and >2.15 mmol/L at 2 DIM and for multiparous Ca ≤1.77 at 1 DIM and >2.20 mmol/L at 4 DIM were defined as tSCH. The second group is persistent SCH (pSCH), which was defined as Ca ≤2.15 mmol/L at 1 and 2 DIM for primiparous and Ca ≤1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM for multiparous cows. The third group is delayed SCH (dSCH), which is considered Ca >2.15 at 1 DIM and ≤2.15 mmol/L at 2 DIM for primiparous and Ca >1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM for multiparous cows.

3.2.4 *Hypocalcemia management and prevention*

Different practices have been proposed to not only provide effective and timely treatment to cows suffering hypocalcemia but also to prevent this prevalent disease. One of the strategies that has been suggested by the older literature is to reduce the number of absorbable dietary cations and/or increase the number of absorbable dietary anions (Block, 1984; Ender et al., 1971). Reportedly, Na (+1), K (+1), Ca (+2), and Mg (+2) are the most common cations present in common feed stuff utilized to in cattle diets, while Cl (−1), SO4 (−2), and phosphate (−3) are the most prevalent anions (Goff, 2008). Dishington (1975) first reported that the prepartum feeding of a mixture of chloride and sulfate salts could significantly decrease the incidence of CH. It is conceived that the general acid–base balance of the body and the pH of the blood could be determined by the difference between the number of cation and anion particles absorbed from the diet (Goff, 2008). Lowering dietary cation-anion difference (DCAD) before calving has been shown to reduce the risk for clinical and subclinical hypocalcemia (Charbonneau et al., 2006).

Another preventive approach focused on lowering the chances of developing hypocalcemia is oral or intravenous Ca supplementation immediately after calving (Blanc et al., 2014). Oetzel & Miller (2012) evaluated the effects of oral Ca supplementation after calving on early-lactation health and milk yield in MULT cows and reported positive effects on lame and high milk producing cows. Additionally, Martinez et al. (2016) reported that oral Ca supplementation reduced the incidence of subclinical hypocalcemia. In the latter study, authors reported that Ca treatment increased incidence of metritis and morbidity rate in primiparous cows. They concluded that Ca administration would be beneficial only in primiparous cows that are at a higher risk of developing hypocalcemia, and large doses of oral Ca in the first days postpartum should be avoided in normal primiparous cows. Similarly, in another study conducted by this lab (Martinez et al., 2016), it was shown that oral Ca supplementation benefited reproductive performance in multiparous cows and improves milk yield in the cohort of multiparous cows with greater production potential. Similarly to the previous study, authors reported negative effects in primiparous cows, this time in reproductive performance.

According to Domino et al. (2017), who decided to assess the effects of both oral and subcutaneous Ca supplementation on different post-partum diseases and milk production, serum Ca concentrations is increased by both Ca supplementation strategies; however, no significant effects were observed on milk production, health or reproductive outcomes. Valldecabres et al. (2018) supplemented a group of postpartum Jersey cows with oral Ca boluses and reported higher serum Ca concentration in this group compared to the control group. In an interesting study by Jahani-Moghadam et al. (2020), it was reported that oral Ca supplementation after calving might be preferred compared to subcutaneous injection.

Chapter 3

Effects of postpartum acetylsalicylic acid administration and calcium supplementation on metabolism, inflammation, health, production, reproductive performance, and culling in multiparous Holstein dairy cows

Abstract

The objective of this study was to assess effects of the combination of postpartum acetylsalicylic acid administration and calcium supplementation on haptoglobin concentration (Hp), calcium (Ca) concentration, beta-hydroxybutyrate (BHB), and the prevalence of subclinical ketosis, subclinical hypocalcemia, and clinical metritis in multiparous (≥2 lactation) cows. Within 12 h after calving, cows were randomly allocated to one of four treatment groups: 1) ASA (n=155) = Cows received two oral administrations with ASA 24 h apart (125 g/cow/d; 4 480-grain aspirin boluses); 2) ASACAL (n=164) = Cows received two oral administrations with ASA (125 g/cow/d; 4 480-grain aspirin boluses) and Ca (43 g/cow/d; 2 Ca boluses) 24 h apart, 3) CAL (n=171) = cows received two oral administrations with Ca (43 g/cow/d; 2 Ca boluses) 24 h apart, and 4) UNT (n=156) = cows remained untreated. Blood samples were collected before each treatment and at 7±3 DIM to assess Ca, Hp, and BHB concentrations. Cows that presented a BHB concentration of >1.2 mmol/L were classified as having subclinical ketosis, while a Ca concentration <8 mg/dL was classified as subclinical hypocalcemia. Vaginal discharge was scored weekly from calving until 21±3 DIM for assessment of clinical metritis, defined as the presence of a brownish fetid vaginal discharge. On-farm records were collected to assess health events in the first 60 DIM, DHIA test data, reproductive performance, and culling for 300 DIM. The data were analyzed using MIXED, GLIMMIX, and LIFETEST SAS procedures. There was no difference in BHB or Hp concentrations between treatment groups. Cows that became sick in the first 60 DIM had lower concentrations of Ca and higher concentrations of BHB and Hp in the first week after calving compared to cows that remained healthy. Intriguingly, cows treated with ASA or ASACAL tended ($p=0.09$) to

have lower concentrations of Ca at the second treatment administration compared to UNT (ASA= 7.66±0.07 mg/dL; ASACAL= 7.64±0.06 mg/dL; UNT= 7.90±0.07 mg/dL). Cows treated with CAL tended (p=0.1) to have a lower prevalence of subclinical ketosis compared to UNT cows (CAL=36.96±4.95%; UNT=46.63±5.19%). On the other hand, cows treated with ASA tended (p=0.1) to have a lower incidence of clinical metritis compared to CAL cows (ASA=15.92±3.56%; CAL=29.92±4.40%). Regarding DHIA fat-corrected milk, there was a test*treatment*parity interaction (*p*=0.03). Second lactation ASACAL cows produced 1.54 kg, 3.4 kg, 1.45 kg, and 1.27 kg less fat-corrected milk in DHIA tests 1, 2, 4, and 5, respectively, compared to second lactation UNT cows. Conversely, third+ lactation ASACAL cows produced 3.42 kg and 3.53 kg more fat-corrected milk in DHIA tests 2 and 3, respectively, compared to third+ lactation UNT cows. Similarly, second lactation ASACAL cows tended to produce 537 kg less ME305 milk compared to second lactation UNT cows, while third+ lactation ASACAL cows tended to produce 443 kg more ME305 milk than third+ lactation UNT cows. ASACAL cows tended to require fewer days (*p*=0.10; ASACAL=101.77±4.18 d; UNT=111.45±4.13 d) and fewer services (*p*=0.05; ASACAL= 1.91±0.13 svc.; UNT=2.27±0.13 svc.) to become pregnant compared to UNT cows. Interestingly, third+ lactation ASAPCAL cows had a lower fat percentage in the DHIA test 1 compared to third+ lactation UNT cows. These findings suggest that postpartum acetylsalicylic acid administration alone or in combination with Ca supplementation may alter Ca metabolism. Furthermore, these results strengthen the existent knowledge regarding the adverse effects of postpartum inflammation and poor Ca and fat tissue metabolism on cow health. In addition, postpartum acetylsalicylic acid combined with Ca may improve milk yield in third+ lactation cows, while it may be

detrimental for second-lactation cows. Moreover, ASACAL treatment might positively affect fertility in multiparous cows.

Keywords: Postpartum period, Calcium and acetylsalicylic acid, Cow metabolism and health

Introduction

The transition period (TP) which is confined to three weeks before and three weeks after calving is the most physically, physiologically, and metabolically challenging period for dairy cows as they are passaging through early lactation from late gestation (Goff & Horst, 1997; Grummer, 1995). An efficient transition into lactation is essential to ensure the success of dairy cows in current production systems due to a wide range of long-term effects which are associated with transition management (Drackley, 1999). As the nutrient requirements of the fetus reach maximal levels prior to calving, dry matter intake (DMI) continuously decreases during the last 15 to 10 days pre-partum and is 20 – 40% lower at the day of parturition (Martens, 2023). These higher demands for nutrients continue to rise after calving with the onset of lactogenesis, exceeding the energy intake levels (Rastani et al., 2005; van Knegsel et al., 2014). It has been reported that in early lactation, energy demands increase by about 300%, and calcium requirements are increased more than 65% to support lactogenesis (Bell, 1995; DeGaris & Lean, 2008; Drackley, 1999). The dramatic drop in DMI, which is accompanied by additional factors such as stress and systemic inflammation, along with the elevated energy and nutrient demands will predispose cow to go through a stage known as negative energy balance (NEB). Under NEB, the cow physiologically mobilizes adipose reserves to meet these requirements and maintain a high level of milk production. Consequently, it has been shown that as a result of this fat mobilization the cow might lose about 60% or more of their body fat in the first weeks after calving (Kadokawa & Martin, 2006). However, the mobilization of body reserves to compensate for this energy deficit is associated with altered metabolic status, greater incidence of metabolic diseases such as ketosis, displaced abomasum, mastitis and decreased fertility (Collard et al., 2000; van Knegsel et al., 2014).

Concurrent with the drop in DMI and NEB, periparturient cows will experience systemic inflammation (SI). As the parturition process approaches, a well-regulated postpartum (POSTP) uterine inflammatory response is highly demanded for various reasons and purposes including pathogens elimination, resumption of the ovarian function, and prevention of reproductive disease (Cheong et al., 2017; Sheldon et al., 2019). Postpartum SI is also considered to be associated with altered metabolism and a series of contributing causes, which includes excessive fat mobilization, leading to pro-inflammatory cytokines production, including tumor necrosis factor alpha (TNF)-α, and interleukin (IL)-6 (Bradford et al., 2015; Trevisi et al., 2012). These cytokines heighten insulin insensitivity and intensify the release, from that dipose tissue, of non-esterified fatty acids (NEFA) as they inhibit intracellular signaling of insulin (De Koster et al., 2018). Proinflammatory cytokines also stimulate the production of acute phase proteins such as haptoglobin (HP), which is commonly used as a biomarker of inflammation (Eckersall & Bell, 2010; Huzzey et al., 2011; McCarthy et al., 2016). Altogether, peripartum SI is aimed at assisting the body to adapt more efficiently and overcome adverse effects during this period with the ultimate goal of restoring homeostasis (Bradford et al., 2015). However, like other physiological processes, if SI becomes exacerbated or prolonged, it can negatively impact the health and performance of the cows. It has been shown that uncontrolled SI is associated with an increased risk of experiencing POSTP health issues that results in defective productivity and reproductive performance in the following lactation (Leblanc, 2010; McArt et al., 2013; Roche et al., 2013). These negative effects clearly highlight the importance of practices and strategies that focused on modulating the inflammatory response throughout the peripartum period.

Another essential adaptation in cow metabolism during the TP is calcium (Ca) homeostasis, which is necessary for colostrum and milk synthesis. In normal situations, Ca is sufficiently absorbed by the body through the diet and it is stored mainly in the bones (Martín-Tereso & Martens, 2014). As previously mentioned, with the onset of lactation, dairy cows experience a marked increase in Ca requirements to support colostrum and milk synthesis (DeGaris & Lean, 2008; Horst et al., 2005). It has been reported that calcium cow needs increase up to six times in the first 24 hours after calving (Couto Serrenho et al., 2021). This dramatic increase in Ca utilization and requirement increases the odds of developing clinical (CH) or subclinical (SCH) hypocalcemia. Hypocalcemia mostly occurs within 12 to 24 h after calving and in its clinical presentation is accompanied by clinical signs that can last for several hours and often Ca concentrations <5.5 mg/dl (Horst et al., 2005). The more concerning challenge; however, is SCH as it remains a prevalent metabolic disorder affecting around 50% of multiparous animals in the U.S. (Reinhardt et al., 2011). According to McArt & Neves (2020), there are three different categories of SCH. The first group is characterized as transient SCH (tSCH) with Ca ≤1.77 at 1 DIM and >2.20 mmol/L at 4 DIM for multiparous cows. The second group is persistent SCH (pSCH), which is defined as Ca ≤1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM for multiparous cows. The third group is known as delayed SCH (dSCH), which is highlighted as Ca >1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM for multiparous cows. Subclinical hypocalcemia has been shown to be associated with gut motility, higher DA risk, decreased milk production performance, increased susceptibility to infectious disease, poor reproductive performance, and an overall higher culling risk (Caixeta et al., 2017; Curtis et al., 1984; Hansen et al., 2003; Oetzel & Miller, 2012; Seifi et al., 2011), causing to the dairy industry millions of dollar in losses.

One of the management strategies that has been suggested to modulate POSTP inflammation is anti-inflammatory treatments. In this regard, use of nonsteroidal anti-inflammatory drugs (NSAIDs) as one of the most common family of anti-inflammatory drugs has been widely studied. While a number of treatment approaches (e.g., number of doses, type of administration) utilizing a variety of NSAIDs (e.g., carprofen, meloxicam) have been studied, results have been highly inconsistent (Bertoni et al., 2004; Farney et al., 2013; Giammarco et al., 2018; Meier et al., 2014; Newby et al., 2017; Swartz et al., 2018).

Among the wide variety of NSAIDs, administration of acetylsalicylic acid (ASA) commonly known as aspirin after calving has been shown to have consistently positive effects on cow performance and health (e.g., increased milk yield, improved fertility) in multiparous cows (Barragan et al., 2020a; Barragan et al., 2020b; Bertoni et al., 2004; Carpenter et al., 2016; Trevisi and Bertoni, 2008; Farney et al., 2013). However, the synergistic effects of combining these anti-inflammatory strategies with hypocalcemia preventative strategies have not been assessed yet.

As one of the other important management practices, Ca supplementation after calving has been shown to improve cow metabolism and performance in multiparous cows (Martinez et al., 2014; Martinez et al., 2016a; Martinez et al., 2016b). For instance, Martinez et al. (2016a) reported that post-partum oral Ca supplementation decreased subclinical hypocalcemia in multiparous (MULT) cows. Similarly, they reported that MULT cows supplemented with oral Ca had a higher milk yield in the first 30 DIM and improved pregnancy per artificial insemination (AI) at first and all services (Martinez et al., 2016b). Similarly to SI modulation strategies, the possible synergistic benefits of combining Ca supplementation with anti-inflammatory treatment warrants further research. Therefore, the objective of this research was to assess effects of the combination of ASA administration and Ca supplementation after calving on metabolism (i.e., circulating haptoglobin [Hp] and β-hydroxybutyrate [BHB]; body condition score [BSC]), health (i.e., incidence of clinical disease events), production (i.e., milk yield, milk components, milk quality [SCC]), culling rate and reproductive performance (i.e., DIM to conception, times bred, pregnancy per artificial insemination [AI], abortion rate) in post-partum cows. We hypothesized that the combination of these two disease preventative strategies would have synergistic effects and would maximize health and performance in POSTP MULT Holstein dairy cows.

Materials and Methods

This study was performed on a large commercial dairy farm located in central Pennsylvania, between May 2022 and June 2023. During the study period, this farm milked 875 cows and had a rolling herd average of 28829 pounds. At around 14 days prior to their expected calving date, cows were moved to a freestall deep sand-bedded pen. The personnel at the farm walked this pen at about 1-hour intervals, monitoring animals for signs of calving such as the appearance of the amniotic sac or feet of the calf external to the vulva. Cows showing these signs were moved to a contiguous individual straw-bedded pen to be allowed to calve comfortably and receive properly and in a timely manner assistance in case of developing dystocia. After calving, cows were moved into a continuous loose straw-bedded small pen (15-20 cows) for 3-4 days where expulsion of fetal membranes, as well as any health concern, were closely monitored. Then cows were moved to a larger freestall deep sand-bedded POSTP pen where they remained for around 21 days after calving. All stalls had appropriate heat abatement systems in place (i.e., fans and sprinklers). Animals were offered a TMR diet twice daily and milked three times per day. Total mixed rations were formulated to meet or exceed the dietary nutritional requirements of high-producing lactating cows (NASEM, 2021). Additionally, farm personnel performed feed push-ups every ~2-3 hours. The Institutional Animal Care and Use Committee (IACUC) at the Pennsylvania State University approved all the procedures listed below the procedures.

Animal enrollment

Based on previous literature findings regarding the association of ASA treatment and Ca supplementation benefits with cow parity (i.e., MULT cows benefited from treatment while primiparous cows were negatively affected or not affected), only MULT cows were included in this study. Furthermore, cows that experienced severe calving assistance (i.e., c-section or fetotomy) were not included in the study.

A total of 667 animals were enrolled which out of those, 8 were removed due to receiving improper treatment (i.e., receiving more than two boluses or receiving the treatment in a different way of administration) and 13 were removed as they were sold or died within the first 7±3 DIM and did not have enough samples. Within 12 hours after parturition, post-parturient MULT dairy cows were randomly allocated to one of the four treatment groups: 1) ASA (n = 155) = Cows received 2 oral administrations with aspirin 24 h apart (125 g/cow/d; 4 480- grain aspirin boluses; Agri Labs, St. Joseph, MO), 60 ASA cows were second lactation and 95 were third+ lactation, 2) ASACAL (n = 164) = Cows received 2 oral administrations with aspirin (125 g/cow/d; 4 480-grain aspirin boluses; Agri Labs, St. Joseph, MO) and calcium (43 g/cow/d; 2 calcium boluses; Bovikalc, Boehringer Ingelheim, St. Joseph, MO) 24 h apart, 57 ASACAL cows were second lactation and 107 were third+ lactation, 3) CAL (n = 171) = Cows received 2 oral administrations with calcium (43 g/cow/d; 2 calcium boluses; Bovikalc, Boehringer Ingelheim, St. Joseph, MO) 24 h apart, 60 CAL cows were second lactation and 111 were third+ lactation, and 4) UNT (n = 156) = Cows remained untreated as a negative control group, 69 UNT cows were second lactation and 87 were third+ lactation. All treatments were performed by farm personnel (n=2), who were trained by the research team prior to the study onset. Briefly, treatments were performed twice a day (i.e., morning - starting at 0900–1000 h, afternoon - starting at 1700–1800 h; Barragan et al., 2020).

Blood sample collection, clinical metritis assessment and body condition scoring

Blood samples were collected immediately before each treatment (i.e., 12h POSTP, 24h POSTP) and then weekly for the first three weeks after calving (i.e., 7±3, 14±3, and

21±3 DIM) through coccygeal bleeding using 10 mL vacutainer tubes. Samples were stored in a portable cooler containing ice packs after collection until returning to the lab for further analysis. Clinical metritis (CM) was checked for all the enrolled cows with the Metricheck® device at 7±3, 14±3, and 21±3 days after calving. Clinical metritis was defined as presence of a watery red-brownish fluid to viscous, purulent, and fetid vaginal discharge (Noakes et al., 2019). Body Condition Score assessment was performed weekly using a 5-point scale (Ferguson et al., 1994) starting at 7±3 until 21±3 DIM, using a 0.25-points increments by 3 members of research team personnel.

Blood sample processing and blood metabolite assessment

Blood samples were centrifuged (15 min at 1,500 x g at room temperature 25ºC) to aliquot and transfer the serum to 2 mL tubes within 2 h after the collection. Samples were stored in duplicate at -20ºC freezers until further analysis. Haptoglobin concentrations were assessed in the first three samples (i.e., 12h POSTP, 24h POSTP, 7±3 DIM). The concentration of HP in serum was determined with a commercial bovine haptoglobin ELISA kit (Life Diagnostics, West Chester, PA), as described by Barragan et al. (2020c). The average intra-assay (within the plate) and inter-assay (between plates) coefficients of variation were 4.9% and 5.3% respectively. All samples were analyzed in duplicate. Samples that had a concentration higher than the upper limit of qualification of the assay were diluted and re-analyzed. The serum concentration of BHB was assessed on the samples collected at 7±3, 14±3, and 21±3 DIM using an electronic cow-side test (BHBCheck Plus from PortaCheck, Moorestown, NJ). Animals that had a BHB concentration equal to or greater than 1.2 mmol/L were classified as having subclinical ketosis (LeBlanc, 2010). Similar to HP, tCa concentration measurement was only performed on the first three samples (i.e., 12h POSTP, 24h POSTP, 7±3 DIM). These samples were shipped to the University of Illinois veterinary diagnostic laboratory for tCa analysis. Total Ca concentration of ≤8 mg/dL was classified as subclinical hypocalcemia.

Clinical health events, milk yield and components, culling rate and reproductive performance

Clinical health events during the first 60 DIM were collected from on-farm computer records (Dairy Comp 305, Valley Ag Software, Tulare, CA). Cows were classified in two different groups based on their clinical health status (i.e., HLT=no recorded clinical health events in the first 60 DIM; CHEVT=one or more clinical health events recorded in the first 60 DIM). Daily milk yield from the first 60 DIM and monthly DHIA test data (305-d mature equivalent [305-d ME] milk, fat corrected milk, milk fat percentage, milk protein percentage, and somatic cell count [SCC]) for the first 8 tests were collected from on-farm computer records (Dairy Comp 305, Valley Ag Software, Tulare, CA). Culling rate at 60 DIM as well as reproductive performance data (i.e., Days in Milk to Conception [DIMC], Services per Conception [SPC], Pregnancy at First Service [PFS] and Abortion Rate [ABRT]) for 300 DIM were also collected from on farm computer records.

Statistical analysis

Assuming a power of 80% and at least a difference of 5% in disease incidence (Barragan et al., 2021) between treatment groups, with adequate statistical significance (alpha=0.05), and considering an estimated experimental unit (cow) attrition rate of 20%, a sample size of 150 cows per group (total of 600 animals) was required (Sample Size Calculator GPower 3.1).

The data was analyzed as a randomized complete block design, and statistical analyses were performed using the SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC). The UNIVARIATE procedure of SAS was used to assess the normality and homogeneity of variances (Shapiro-Wilk statistic, graphical methods [histogram and Q-Q plot], and Barlett's tests) for the quantitative variables. Continuous variables were analyzed with the MIXED procedure of SAS. For analysis of BCS, HP, BHB, tCa, daily milk yield, and DHIA tests' data, , the REPEATED statement was included in the MIXED procedure. The covariate structures were selected using the best fit according to Schwarz's Bayesian information criteria. Selection of the variables that remained in the model was performed using the Wald statistic backward selection criterion ($P > 0.15$). The variable cow was included in the RANDOM statement of the MIXED procedure. The variable treatment (TRT), day, and day*TRT were forced in all the models. The results are presented as least squares means (LSM) and standard error of the mean (SEM), calculated and adjusted with Tukey-Kramer method using the LSMEAN statement. When the interactions between TRT and day were significant ($P < 0.05$), the 'slice' option in the 'lsmeans' statement was used to determine differences among the treatments on each day. The variables BHB were log10 transformed due to a lack of normality. Results from these two variables were backtransformed, and geometric means and 95% confidence intervals were presented.

The culling rate and binomial reproductive performance variables were assessed using multivariable logistic regression models generated by the GLIMMIX procedure of SAS. To assess TRT effects on DIMC and culling rate, the LIFETEST procedure of SAS was used to assess statistical association and obtain data output to develop a survival curve graphic in Excel (Microsoft Corp., Redmond, WA) of statistically significant outcomes to depict the proportion of cows pregnant up to 300 DIM. The main variables of interest and their interactions were considered statistically significant if $P < 0.05$, and $0.05 < P < 0.10$ was considered a tendency.

Results

β-hydroxybutyrate, haptoglobin and total calcium concentration

There was no difference in BHB concentration during the first 21 DIM between treatment groups (ASA=0.98 mmol/L, CI 95%=0.06-0.07; ASACAL=0.91 mmol/L, CI 95%=0.06-0.06; CAL= 0.96 mmol/L, CI 95%=0.06-0.06; UNT=1.00 mmol/L, CI 95%=0.06-0.07; p=0.16). Similarly, Hp concentrations before each treatment administration and at 7±3 DIM was not different between treatment groups (ASA=112.24±4.92 µg/mL; ASACAL=112.93±4.70 µg/mL; CAL=105.23±4.67 µg/mL; UNT=105.13±4.68 µg/mL; p=0.26). Cows that became sick in the first 60 DIM had lower concentrations of tCa and higher concentrations of BHB and Hp in the first week after calving compared to cows that remained healthy. Intriguingly, cows treated with ASA or ASACAL tended (p=0.09) to have lower concentrations of tCa at the second treatment administration compared to UNT cows (ASA= 7.66±0.07 mg/dL; ASACAL= 7.64±0.06 mg/dL; UNT= 7.90±0.07 mg/dL).

Subclinical diseases and clinical health events

Cows treated with CAL tended (*p*=0.1) to have a lower incidence of subclinical ketosis compared to UNT cows (CAL=36.96±4.95%; UNT=46.63±5.19%; Table 1). On the other hand, cows treated with ASA tended (p=0.1) to have a lower incidence of clinical metritis compared to CAL cows (ASA=15.92±3.56%; CAL=29.92±4.40%; Table 1). There was no difference in the percentage of animals that had clinical health events during the first 60 DIM between treatment groups (ASA=25.96±5.35%; ASACAL=24.82±4.9%; CAL=24.94±4.87%; UNT=22.62±4.6%; *p*=0.95; Table 1).

Reproductive performance, culling rate and milk yield and components

Cows treated with ASACAL tended to require fewer days (p=0.10; ASACAL=101.77±4.18 d; UNT=111.45±4.13 d) and fewer services (p=0.05; ASACAL= 1.91±0.13 svc.; UNT=2.27±0.13 svc.) to become pregnant compared to UNT cows. However, when assessed using LIFETEST procedure there was no significant difference in DIMC by 150 DIM between ASACAL and UNT cows (*p*=0.17; Figure 2). There was no difference in culling rate between treatment groups (ASA=29.06±3.71%; ASACAL=28.52±3.60%; CAL=24.52±3.33%; UNT=24.67±3.49 %; *p*=0.68; Table 1).

Regarding DHIA fat-corrected milk, there was a test by treatment by parity interaction (p=0.03). Second-lactation cows that received ASACAL treatment produced 1.54 kg, 3.4 kg, 1.45 kg, and 1.27 kg less fat-corrected milk in DHIA tests 1, 2, 4, and 5, respectively, compared to second-lactation UNT cows (Figure 1). Conversely, third+ lactation cows treated with ASACAL produced 3.42 kg and 3.53 kg more fat-corrected milk in DHIA tests 2 and 3, respectively, compared to third+ lactation UNT cows (Figure 1). Similarly, there was a tendency (p=0.05) for an interaction between treatment and parity in ME305, where second lactation ASACAL cows produced 537 kg less milk compared to second-lactation UNT cows, while third+ lactation ASACAL cows produced 443 kg more milk than third+ lactation UNT cows. Interestingly, third+ lactation ASAPCAL cows had a lower fat percentage in the DHIA test 1 compared to third+ lactation UNT cows. There was no difference on milk protein percentage and SCC between treatment groups.

Discussion

The main results of this study were: 1) Postpartum treatment with ASA or ASA combined with Ca administration may negatively affect tCa concentration, 2) The ASACAL treatment approach improve milk yield in 3+ lactation cows but impaired production in second lactation cows, and 3) Regardless of cow lactation, ASACAL may have positive effects in cow reproductive performance.

At the onset of lactation, high amounts of Ca are required for milk production as colostrum and milk contain 1.7 – 2.3 and 1.1 g/L of Ca, respectively (Goff, 2004). This interruption in Ca concentrations has been shown to be associated with a number of health events. For instance, it has been shown concentrations of plasma calcium are lower in cows with retained fetal membranes within 24 h after parturition and during the first week post-partum (Risco et al., 1994). Also, it has been reported that at the time of calving, not only the proportion of the hypocalcemic cows experiencing metritis is higher (25.1% vs. 14.7%) compared to the non hypocalcemic cows, but also the culling rate within 60 DIM is higher (15.9% vs. 6.8%) in that group (Wilhelm et al., 2017). In a study conducted by Venjakob et al. (2021), multiparous cows with either only CH or CH and at least another health event had significantly reduced serum Ca concentrations on 0, 1, 3, and 7 DIM compared with healthy cows. Similarly, multiparous cows with either only ketosis or ketosis and at least another health event were shown to have decreased serum Ca concentrations on d 1 and 3 when compared with healthy cows. This trend was the same in the cows with either only mastitis or mastitis and at least another health event where multiparous cows with mastitis had a decreased serum Ca concentration on d 1, and the latter had a decreased Ca serum concentration on d 1 and 3, compared with healthy multiparous cows. Although in the present study treatment groups with ASA had lower tCa concentrations before the second treatment administration, there was no difference in tCa concentration between treatment groups by 7±3 DIM. Further research is needed to understand the effects of ASA in Ca metabolism and the associations with future health and performance.

Farney et al. (2013) studied effects of the administration of sodium salicylate (SS) in the first week of lactation was assessed on the whole-lactation productivity. Authors reported that there was a treatment by parity for 305-d milk and milk fat yields, and there was a tendency for an interaction for 305-d milk protein yield. Total milk yield, milk fat, and 305-d milk protein yield were increased by 21% (2,469 ± 646 kg), 30% (130 ± 23 kg) and 14%, respectively over the whole lactation period in third+ SS cows compared with third+ control cows, while no effects were detected in the second lactation cows. According to (Oetzel & Miller, 2012) oral Ca supplementation after calving would positively affect high producing cows. On the same lines, Martinez et al. (2016) reported positive effects of oral Ca supplementation after calving in high producing multiparous cows. Seely et al. (2024) reported that delayed oral Ca supplementation (one oral bolus of 43 g of Ca at 48 and 72 h post calving) in third lactation cows will increase milk yield compared to control cows and third lactation cows who received early oral bolus (at time of calving and 24 h later). Combined with the positive effects of post-partum oral Ca supplementation on MULT cows reported by previous studies, the results observed in this study depict the probability of a synergetic effect of the combined treatment on milk yield in third+ lactation cows. However, more research is crucial to establish a more comprehensive understanding of treatment dynamics and the proper timing of the treatment.

Regarding the observed positive effects of ASACAL treatment on reproductive performance regardless of parity, Martinez et al. (2016) reported beneficial effects of post-partum oral Ca supplementation on reproduction in MULT cows. On the other hand, positive effects of post-partum ASA administration on reproductive performances was shown in the Barragan et al. (2020) study where ASA treated cows tended to require fewer days and fewer services to become pregnant compared to PLC cows. A year later, positive effects of postpartum ASA treatment were reported again in Barragan et al. (2021). In the latter study, ASA treated cows had a better reproductive performance compared to untreated cows. These results along with the observed results of the present study suggest potential positive effects of combined ASA administration and Ca supplementation on reproductive performance but further research is still to be conducted.

Conclusions

These results suggest that postpartum ASA administration and Ca supplementation might improve milk yield in 3+ lactation cows, while it may be detrimental for second-lactation cows. Furthermore, the ASACAL treatment approach might posse positive effect for cow fertility; however, larger studies are needed. Interestingly, ASA treatment alone or in combination with Ca administration negatively affected tCa concentration after first treatment administration. Future research should be focused on uncover the effects of ASA administration in Ca metabolism in postpartum cows.

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Tables and Figures

Table 1. Subclinical diseases and clinical health events in cows treated with acetylsalicylic acid (ASA), ASA and calcium (ASACAL), calcium (CAL) or remained untreated (UNT) after calving. Results are presented as LSM±SEM unless specified otherwise below.

	Treatment[1]				
Variable	**ASA (n = 155)**	**ASACAL (n = 164)**	**CAL (n = 171)**	**UNT (n = 156)**	***P*-value**
Subclinical hypocalcemia[a]	53.57±4.84%	45.15±4.84%	51.11±4.69%	42.22±4.73%	0.19
Subclinical ketosis[b]	37.20±4.48%	35.71±4.37%	33.93±4.26%	42.82±4.62%	0.46
Clinical metritis[c]	15.92±3.56%^{a}	26.03±4.19%^{ab}	29.92±4.40%^{b}	23.03±4.1%^{ab}	0.10
Clinical health events	25.96±5.35%	24.82±4.90%	24.94±4.87%	22.62±4.6%	0.95
Culling rate	29.06±3.71%	28.52±3.60%	24.52±3.33%	24.67±3.49%	0.68

a. Cows that had a serum total calcium concentration of ≤8 mg/dL in either of the 24h or 7±3 days in milk were classified as having subclinical hypocalcemia.
b. Cows that had a serum β-hydroxybutyrate concentration equal to or greater than 1.2 mmol/L during the first 21 days after calving were classified as having subclinical ketosis.
c. Clinical metritis was classified as presence of red brownish watery fetid smelling discharge in the first 21 days in milk.
1. ^{a,b} different letters represent a statistical tendency (0.05>p<0.10).

Figure 1.

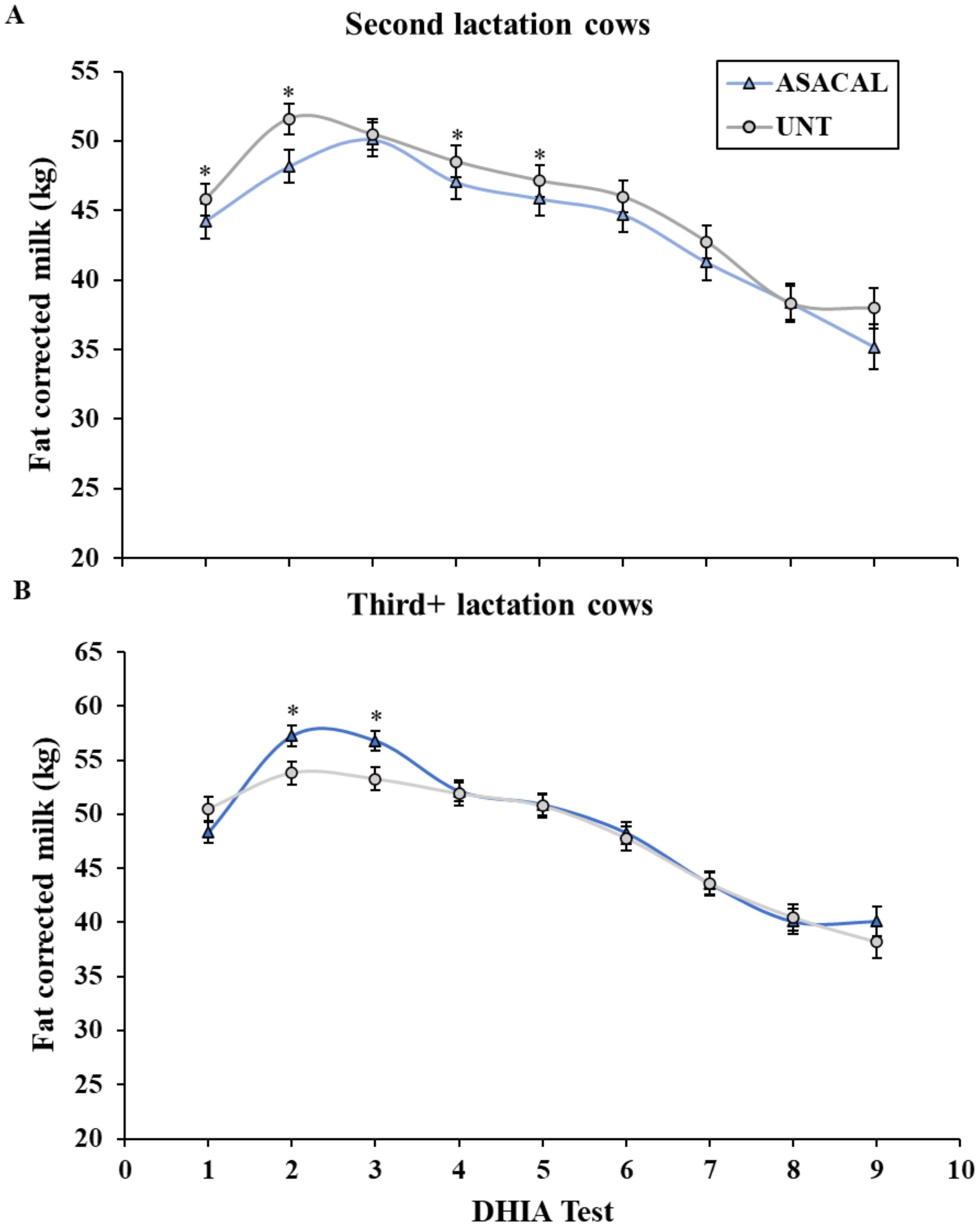
A
Second lactation cows
ASACAL
UNT
Fat corrected milk (kg)
55
50
45
40
35
30
25
20
*
*
*
*
B
Third+ lactation cows
Fat corrected milk (kg)
65
60
55
50
45
40
35
30
25
20
*
*
0
1
2
3
4
5
6
7
8
9
10
DHIA Test

Figure 1. Fat-corrected milk (LSM ± SEM) for the first 9 DHIA tests of (A) second lactation and (B) third+ lactation dairy cows that received postpartum acetylsalicylic acid treatment and calcium administration (ASACAL; n=164) compared with cows that received a untreated animals (UNT; n=156). **p* <0.05. *p*-values are from the “slice” option.

Figure 2.

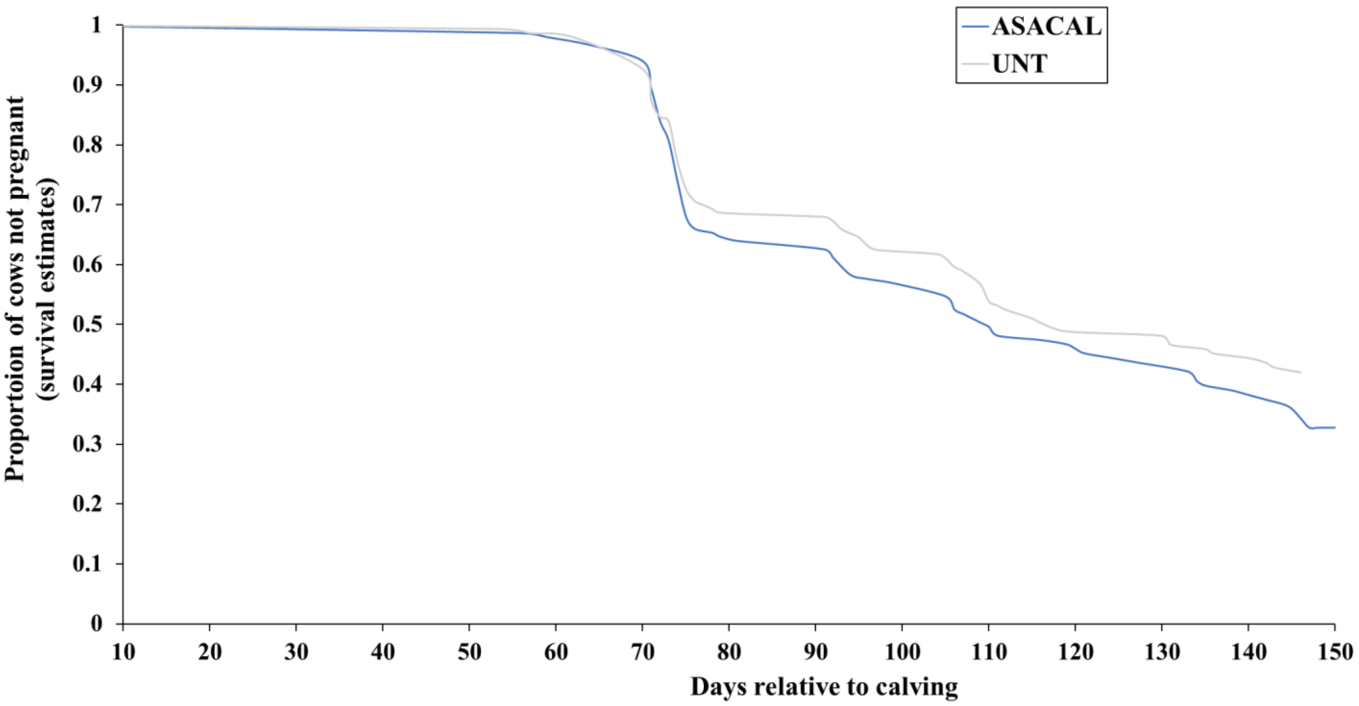

Figure 2. Survival curve for time to pregnancy after the voluntary waiting period of dairy cows treated a combination of postpartum acetylsalicylic acid treatment and calcium administration (ASACAL; n =164) compared with untreated cows (UNT; n=156). Mean time to pregnancy was 111 d (108–114 d) and 114 d (111– 117 d) for ASACAL and UNT cows, respectively (*p*=0.17).

Chapter 4

Conclusions and Future Directions

Based on the results provided by this study, ASA administration and Ca supplementation after calving might potentially improve cow health and metabolism. Furthermore, these results strengthen the known negative effects of postpartum inflammation and poor Ca and fat tissue metabolism on cow health. Additionally, the results illustrated that the combination treatment might improve milk yield in third+ lactation cows, while it may be detrimental for second-lactation cows. Also, ASACAL treatment might positively affect fertility in multiparous cows. Nevertheless, future research seems to be crucial to better understand the mechanisms by which ASA administration and Ca supplementation may cause the observed effects.

The intricate mechanisms involved in the effects of ASA and Ca supplementation after calving warrant further research. Regardless, based on these results, new products that combined these two medications could be developed to increase the applicability of this treatment protocol. This approach would decrease labor associated to treatment administration and improve animal welfare since animal will be restrained for a lower amount of time. However, any product developed would have to be tested to try to replicate the positive effects on this study. Due to some study limitations, this preventative strategy was not evaluated on group of cows with higher risk of developing diseases and having poor performance, such as over conditioned cows and cows that experienced dystocia. Further research is needed to assess the effects of the combination of ASA and Ca administration on this high priority cow groups.

References

1. Akbar, H., Grala, T. M., Vailati Riboni, M., Cardoso, F. C., Verkerk, G., McGowan, J., Macdonald, K., Webster, J., Schutz, K., Meier, S., Matthews, L., Roche, J. R., & Loor, J. J. (2015). Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. *Journal of Dairy Science*, *98*(2), 1019–1032. https://doi.org/10.3168/jds.2014-8584

2. Anderson, K. L., Neff-Davis, C. A., Davis, L. E., & Bass, V. D. (1990). Pharmacokinetics of flunixin meglumine in lactating cattle after single and multiple intramuscular and intravenous administrations. *American Journal of Veterinary Research*, *51*(9), 1464–1467.

3. Atabai, K., Sheppard, D., & Werb, Z. (2007). Roles of the Innate Immune System in Mammary Gland Remodeling During Involution. *Journal of Mammary Gland Biology and Neoplasia*, *12*(1), 37–45. https://doi.org/10.1007/s10911-007-9036-6

4. Bannerman, D. D., Rinaldi, M., Vinyard, B. T., Laihia, J., & Leino, L. (2009). Effects of intramammary infusion of cis–urocanic acid on mastitis-associated inflammation and tissue injury in dairy cows. *American Journal of Veterinary Research*, *70*(3), 373–382. https://doi.org/10.2460/ajvr.70.3.373

5. Barragan, A. A., Bas, S., Hovingh, E., & Byler, L. (2021). Effects of postpartum acetylsalicylic acid on uterine diseases and reproductive performance in dairy cattle. *JDS Communications*, *2*(2), 67–72. https://doi.org/10.3168/jdsc.2020-0047

6. Barragan, A. A., Bauman, L., da Costa, L., Velez, J., Gonzalez, J. D. R., Schuenemann, G. M., Menichetti, B., Piñeiro, J., & Bas, S. (2020). Administration of acetylsalicylic acid after parturition in lactating dairy cows under certified organic management: Part I. Milk yield, milk components, activity patterns, fertility, and health. *Journal of Dairy Science*, *103*(12), 11697–11712. https://doi.org/10.3168/jds.2020-18388

7. Barragan, A. A., Bauman, L. M., Schuenemann, G. M., Velez, J., Lakritz, J., Coetzee, J. F., Gonzalez, J. D. R., Piñeiro, J. M., Menichetti, B., & Bas, S. (2020). Administration of acetylsalicylic acid after parturition in lactating dairy cows under certified organic management: Part II. Biomarkers of nociception, inflammation, and stress. *Journal of Dairy Science*, *103*(12), 11713–11722. https://doi.org/10.3168/jds.2020-18389

8. Barragan, A. A., Hovingh, E., Bas, S., Lakritz, J., Byler, L., Ludwikowski, A., Takitch, S., Zug, J., & Hann, S. (2020). Effects of postpartum acetylsalicylic acid on metabolic status, health, and production in lactating dairy cattle. *Journal of Dairy Science*, *103*(9), 8443–8452. https://doi.org/10.3168/jds.2019-17966

9. Bekker, A., Kloepping, C., & Collingwood, S. (2018). Meloxicam in the management of post-operative pain: Narrative review. *Journal of Anaesthesiology, Clinical Pharmacology*, *34*(4), 450–457. https://doi.org/10.4103/joacp.JOACP_133_18
10. Bell, A. W. (1995). Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science*, *73*(9), 2804–2819. https://doi.org/10.2527/1995.7392804x
11. Bertoni, G., Trevisi, E., & Piccioli-Cappelli, F. (2004). Effects of Acetyl-salicylate Used in Post-calving of Dairy Cows. *Veterinary Research Communications*, *1*(28), 217–219. https://doi.org/10.1023/B:VERC.0000045410.86004.03
12. Bionaz, M., Trevisi, E., Calamari, L., Librandi, F., Ferrari, A., & Bertoni, G. (2007). Plasma Paraoxonase, Health, Inflammatory Conditions, and Liver Function in Transition Dairy Cows. *Journal of Dairy Science*, *90*(4), 1740–1750. https://doi.org/10.3168/jds.2006-445
13. Blanc, C. D., Van der List, M., Aly, S. S., Rossow, H. A., & Silva-del-Río, N. (2014). Blood calcium dynamics after prophylactic treatment of subclinical hypocalcemia with oral or intravenous calcium. *Journal of Dairy Science*, *97*(11), 6901–6906. https://doi.org/10.3168/jds.2014-7927
14. Block, E. (1984). Manipulating Dietary Anions and Cations for Prepartum Dairy Cows to Reduce Incidence of Milk Fever1. *Journal of Dairy Science*, *67*(12), 2939–2948. https://doi.org/10.3168/jds.S0022-0302(84)81657-4
15. Bradford, B. J., Yuan, K., Farney, J. K., Mamedova, L. K., & Carpenter, A. J. (2015). Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *Journal of Dairy Science*, *98*(10), 6631–6650. https://doi.org/10.3168/jds.2015-9683
16. Brommage, R., & DeLuca, H. F. (1985). Regulation of bone mineral loss during lactation. *American Journal of Physiology-Endocrinology and Metabolism*, *248*(2), E182–E187. https://doi.org/10.1152/ajpendo.1985.248.2.E182
17. Brown, E. M., Katz, C., Butters, R., & Kifor, O. (1991). Polyarginine, polylysine, and protamine mimic the effects of high extracellular calcium concentrations on dispersed bovine parathyroid cells. *Journal of Bone and Mineral Research*, *6*(11), 1217–1226. https://doi.org/10.1002/jbmr.5650061112
18. Brunner, N., Groeger, S., Canelas Raposo, J., Bruckmaier, R. M., & Gross, J. J. (2019). Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe1. *Translational Animal Science*, *3*(1), 84–92. https://doi.org/10.1093/tas/txy102

19. Caixeta, L. S., Ospina, P. A., Capel, M. B., & Nydam, D. V. (2015). The association of subclinical hypocalcemia, negative energy balance and disease with bodyweight change during the first 30 days post-partum in dairy cows milked with automatic milking systems. *The Veterinary Journal*, *204*(2), 150–156. https://doi.org/10.1016/j.tvjl.2015.01.021

20. Caixeta, L. S., Ospina, P. A., Capel, M. B., & Nydam, D. V. (2017). Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows. *Theriogenology*, *94*, 1–7. https://doi.org/10.1016/j.theriogenology.2017.01.039

21. Carpenter, A. J., Ylioja, C. M., Vargas, C. F., Mamedova, L. K., Mendonça, L. G., Coetzee, J. F., Hollis, L. C., Gehring, R., & Bradford, B. J. (2016). Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. *Journal of Dairy Science*, *99*(1), 672–679. https://doi.org/10.3168/jds.2015-10048

22. Challis, J. R., Lockwood, C. J., Myatt, L., Norman, J. E., Strauss, J. F., & Petraglia, F. (2009). Inflammation and Pregnancy. *Reproductive Sciences*, *16*(2), 206–215. https://doi.org/10.1177/1933719108329095

23. Chapinal, N., Carson, M., Duffield, T. F., Capel, M., Godden, S., Overton, M., Santos, J. E. P., & LeBlanc, S. J. (2011). The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science*, *94*(10), 4897–4903. https://doi.org/10.3168/jds.2010-4075

24. Charbonneau, E., Pellerin, D., & Oetzel, G. R. (2006). Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. *Journal of Dairy Science*, *89*(2), 537–548. https://doi.org/10.3168/jds.S0022-0302(06)72116-6

25. Cheong, S. H., Filho, O. G. S., Absalon-Medina, V. A., Schneider, A., Butler, W. R., & Gilbert, R. O. (2017). Uterine and systemic inflammation influences ovarian follicular function in postpartum dairy cows. *PLOS ONE*, *12*(5), e0177356. https://doi.org/10.1371/journal.pone.0177356

26. Collard, B. L., Boettcher, P. J., Dekkers, J. C. M., Petitclerc, D., & Schaeffer, L. R. (2000). Relationships Between Energy Balance and Health Traits of Dairy Cattle in Early Lactation. *Journal of Dairy Science*, *83*(11), 2683–2690. https://doi.org/10.3168/jds.S0022-0302(00)75162-9

27. Contreras, G. A., O'Boyle, N. J., Herdt, T. H., & Sordillo, L. M. (2010). Lipomobilization in periparturient dairy cows influences the composition of plasma nonesterified fatty acids and leukocyte phospholipid fatty acids. *Journal of Dairy Science*, *93*(6), 2508–2516. https://doi.org/10.3168/jds.2009-2876

28. Couto Serrenho, R., DeVries, T. J., Duffield, T. F., & LeBlanc, Stephen. J. (2021). Graduate Student Literature Review: What do we know about the effects of clinical and subclinical hypocalcemia on health and performance of dairy cows? *Journal of Dairy Science*, *104*(5), 6304–6326. https://doi.org/10.3168/jds.2020-19371

29. Curtis, C. R., Erb, H. N., Sniffen, C. J., & Smith, R. D. (1984). Epidemiology of Parturient Paresis: Predisposing Factors with Emphasis on Dry Cow Feeding and Management1. *Journal of Dairy Science*, *67*(4), 817–825. https://doi.org/10.3168/jds.S0022-0302(84)81372-7

30. Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity*, *21*(2), 153–160. https://doi.org/10.1016/j.bbi.2006.09.006

31. De Koster, J., Hostens, M., Van Eetvelde, M., Hermans, K., Moerman, S., Bogaert, H., Depreester, E., Van den Broeck, W., & Opsomer, G. (2015). Insulin response of the glucose and fatty acid metabolism in dry dairy cows across a range of body condition scores. *Journal of Dairy Science*, *98*(7), 4580–4592. https://doi.org/10.3168/jds.2015-9341

32. De Koster, J., Nelli, R. K., Strieder-Barboza, C., de Souza, J., Lock, A. L., & Contreras, G. A. (2018). The contribution of hormone sensitive lipase to adipose tissue lipolysis and its regulation by insulin in periparturient dairy cows. *Scientific Reports*, *8*(1), 13378. https://doi.org/10.1038/s41598-018-31582-4

33. Dechow, C. D., Rogers, G. W., Sander-Nielsen, U., Klei, L., Lawlor, T. J., Clay, J. S., Freeman, A. E., Abdel-Azim, G., Kuck, A., & Schnell, S. (2004). Correlations Among Body Condition Scores from Various Sources, Dairy Form, and Cow Health from the United States and Denmark. *Journal of Dairy Science*, *87*(10), 3526–3533. https://doi.org/10.3168/jds.S0022-0302(04)73489-X

34. DeGaris, P. J., & Lean, I. J. (2008). Milk fever in dairy cows: A review of pathophysiology and control principles. *The Veterinary Journal*, *176*(1), 58–69. https://doi.org/10.1016/j.tvjl.2007.12.029

35. Dishington, I. W. (1975). Prevention of Milk Fever (Hypocalcemic Paresis Puerperalis) by Dietary Salt Supplements. *Acta Veterinaria Scandinavica*, *16*(4), 503–512. https://doi.org/10.1186/BF03546643

36. Domino, A. R., Korzec, H. C., & McArt, J. A. A. (2017). Field trial of 2 calcium supplements on early lactation health and production in multiparous Holstein cows. *Journal of Dairy Science*, *100*(12), 9681–9690. https://doi.org/10.3168/jds.2017-12885

37. Drackley, J. K. (1999). Biology of Dairy Cows During the Transition Period: The Final Frontier? *Journal of Dairy Science*, *82*(11), 2259–2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3

38. Bertoni, Trevisi, E. (2008). Attenuation with acetylsalicylate treatments of inflammatory conditions in periparturient dairy cows. *Aspirin and Health Research Progress*, 22–37.

39. Eckersall, P. D., & Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal*, *185*(1), 23–27. https://doi.org/10.1016/j.tvjl.2010.04.009

40. Ender, F., Dishington, I. W., & Helge-bostad, A. (1971). Calcium balance studies in dairy cows under experimental induction and prevention of hypo-calcaemic paresis puerperalis. The solution of the aetiology and the prevention of milk fever by dietary means. *Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde*, *28*(5), 233–256.

41. Engelhardt, G. (1996). Pharmacology of Meloxicam, A New Non-Steroidal Anti-Inflammatory Drug with an Improved Safety Profile Through Preferential Inhibition of COX-2. *Rheumatology*, *35*(suppl_1), 4–12. https://doi.org/10.1093/rheumatology/35.suppl_1.4

42. Erb, H. N., Smith, R. D., Oltenacu, P. A., Guard, C. L., Hillman, R. B., Powers, P. A., Smith, M. C., & White, M. E. (1985). Path Model of Reproductive Disorders and Performance, Milk Fever, Mastitis, Milk Yield, and Culling in Holstein Cows. *Journal of Dairy Science*, *68*(12), 3337–3349. https://doi.org/10.3168/jds.S0022-0302(85)81244-3

43. Farney, J. K., Mamedova, L. K., Coetzee, J. F., Minton, J. E., Hollis, L. C., & Bradford, B. J. (2013). Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. *Journal of Dairy Science*, *96*(12), 7709–7718. https://doi.org/10.3168/jds.2013-7088

44. Furken, C., Nakao, T., & Hoedemaker, M. (2015). Energy balance in transition cows and its association with health, reproduction and milk production. *Tierärztliche Praxis Ausgabe G: Großtiere / Nutztiere*, *43*(6), 341–349. https://doi.org/10.15653/TPG-150371

45. Gao, W., Du, X., Lei, L., Wang, H., Zhang, M., Wang, Z., Li, X., Liu, G., & Li, X. (2018). NEFA-induced ROS impaired insulin signalling through the JNK and p38MAPK pathways in non-alcoholic steatohepatitis. *Journal of Cellular and Molecular Medicine*, *22*(7), 3408–3422.

46. Garrett, J. E., Tamir, H., Kifor, O., Simin, R. T., Rogers, K. V., Mithal, A., Gagel, R. F., & Brown, E. M. (1995). Calcitonin-secreting cells of the thyroid express an

extracellular calcium receptor gene. *Endocrinology*, *136*(11), 5202–5211. https://doi.org/10.1210/endo.136.11.7588259

47. Giammarco, M., Fusaro, I., Vignola, G., Manetta, A. C., Gramenzi, A., Fustini, M., Palmonari, A., & Formigoni, A. (2018). Effects of a single injection of Flunixin meglumine or Carprofen postpartum on haematological parameters, productive performance and fertility of dairy cattle. *Animal Production Science*, *58*(2), 322–331. https://doi.org/10.1071/AN16028
48. Goff, J. P. (2004). Macromineral disorders of the transition cow. *Veterinary Clinics: Food Animal Practice*, *20*(3), 471–494. https://doi.org/10.1016/j.cvfa.2004.06.003
49. Goff, J. P. (2008). The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. *The Veterinary Journal*, *176*(1), 50–57. https://doi.org/10.1016/j.tvjl.2007.12.020
50. Goff, J. P., & Horst, R. L. (1997). Physiological Changes at Parturition and Their Relationship to Metabolic Disorders1, 2. *Journal of Dairy Science*, *80*(7), 1260–1268. https://doi.org/10.3168/jds.S0022-0302(97)76055-7
51. Goff, J. P., Horst, R. L., Jardon, P. W., Borelli, C., & Wedam, J. (1996). Field Trials of an Oral Calcium Propionate Paste as an Aid to Prevent Milk Fever in Periparturient Dairy Cows1. *Journal of Dairy Science*, *79*(3), 378–383. https://doi.org/10.3168/jds.S0022-0302(96)76375-0
52. Gordon, J. L., LeBlanc, S. J., & Duffield, T. F. (2013). Ketosis Treatment in Lactating Dairy Cattle. *Veterinary Clinics: Food Animal Practice*, *29*(2), 433–445. https://doi.org/10.1016/j.cvfa.2013.03.001
53. Gøtzsche, P. C. (2010). NSAIDs. *BMJ Clinical Evidence*, *2010*, 1108.
54. Graugnard, D. E., Bionaz, M., Trevisi, E., Moyes, K. M., Salak-Johnson, J. L., Wallace, R. L., Drackley, J. K., Bertoni, G., & Loor, J. J. (2012). Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum1. *Journal of Dairy Science*, *95*(4), 1749–1758. https://doi.org/10.3168/jds.2011-4579
55. Gross, J., van Dorland, H. A., Bruckmaier, R. M., & Schwarz, F. J. (2011). Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. *Journal of Dairy Science*, *94*(4), 1820–1830. https://doi.org/10.3168/jds.2010-3707
56. Gruber, S., & Mansfeld, R. (2019). Herd health monitoring in dairy farms – discover metabolic diseases. An overview. *Tierärztliche Praxis Ausgabe G: Großtiere / Nutztiere*, *47*(04), 246–255. https://doi.org/10.1055/a-0949-1637

57. Grummer, R. R. (1995). Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science*, *73*(9), 2820–2833. https://doi.org/10.2527/1995.7392820x

58. Grummer, R. R., Mashek, D. G., & Hayirli, A. (2004). Dry matter intake and energy balance in the transition period. *Veterinary Clinics: Food Animal Practice*, *20*(3), 447–470. https://doi.org/10.1016/j.cvfa.2004.06.013

59. Hansen, S. S., Blom, J. Y., Ersbøll, A., & Jørgensen, R. J. (2003). Milk fever control in Danish dairy herds. *Acta Veterinaria Scandinavica Supplementum*, *97*, 137–139.

60. Herdt, T. H. (2000). Ruminant Adaptation to Negative Energy Balance: Influences on the Etiology of Ketosis and Fatty Liver. *Veterinary Clinics of North America: Food Animal Practice*, *16*(2), 215–230. https://doi.org/10.1016/S0749-0720(15)30102-X

61. Hernández-Castellano, L. E., Hernandez, L. L., & Bruckmaier, R. M. (2020). Review: Endocrine pathways to regulate calcium homeostasis around parturition and the prevention of hypocalcemia in periparturient dairy cows. *Animal*, *14*(2), 330–338. https://doi.org/10.1017/S1751731119001605

62. Hoenderop, J. G. J., Nilius, B., & Bindels, R. J. M. (2005). Calcium Absorption Across Epithelia. *Physiological Reviews*, *85*(1), 373–422. https://doi.org/10.1152/physrev.00003.2004

63. Horst, E. A., Kvidera, S. K., & Baumgard, L. H. (2021). Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas. *Journal of Dairy Science*, *104*(8), 8380–8410. https://doi.org/10.3168/jds.2021-20330

64. Horst, R. L., Goff, J. P., & McCluskey, B. J. (2006). Prevalence of subclinical hypocalcemia in U.S. dairy operations. In *Production diseases in farm animals* (pp. 215–215). Wageningen Academic. https://doi.org/10.3920/9789086865710_068

65. Horst, R. L., Goff, J. P., & Reinhardt, T. A. (1994). Calcium and Vitamin D Metabolism in the Dairy Cow1. *Journal of Dairy Science*, *77*(7), 1936–1951. https://doi.org/10.3168/jds.S0022-0302(94)77140-X

66. Horst, R. L., Goff, J. P., & Reinhardt, T. A. (2005). Adapting to the Transition Between Gestation and Lactation: Differences Between Rat, Human and Dairy Cow. *Journal of Mammary Gland Biology and Neoplasia*, *10*(2), 141–156. https://doi.org/10.1007/s10911-005-5397-x

67. Huzzey, J. M., Duffield, T. F., LeBlanc, S. J., Veira, D. M., Weary, D. M., & von Keyserlingk, M. A. G. (2009). Short communication: Haptoglobin as an early

indicator of metritis. *Journal of Dairy Science*, *92*(2), 621–625. https://doi.org/10.3168/jds.2008-1526

68. Huzzey, J. M., Nydam, D. V., Grant, R. J., & Overton, T. R. (2011). Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. *Journal of Dairy Science*, *94*(12), 5878–5889. https://doi.org/10.3168/jds.2010-3391

69. Ingvartsen, K. L. (2006). Feeding- and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. *Animal Feed Science and Technology*, *126*(3), 175–213. https://doi.org/10.1016/j.anifeedsci.2005.08.003

70. Jahani-Moghadam, M., Yansari, A. T., Chashnidel, Y., Dirandeh, E., & Mahjoubi, E. (2020). Short- and long-term effects of postpartum oral bolus v. Subcutaneous Ca supplements on blood metabolites and productivity of Holstein cows fed a prepartum anionic diet. *Animal*, *14*(5), 983–990. https://doi.org/10.1017/S175173111900257X

71. Kadokawa, H., & Martin, G. B. (2006). A New Perspective on Management of Reproduction in Dairy Cows: The Need for Detailed Metabolic Information, an Improved Selection Index and Extended Lactation. *Journal of Reproduction and Development*, *52*(1), 161–168. https://doi.org/10.1262/jrd.17088

72. Kelton, D. F., Lissemore, K. D., & Martin, R. E. (1998). Recommendations for Recording and Calculating the Incidence of Selected Clinical Diseases of Dairy Cattle. *Journal of Dairy Science*, *81*(9), 2502–2509. https://doi.org/10.3168/jds.S0022-0302(98)70142-0

73. Knegsel, A. T. M. van, Hammon, H. M., Bernabucci, U., Bertoni, G., Bruckmaier, R. M., Goselink, R. M. A., Gross, J. J., Kuhla, B., Metges, C. C., Parmentier, H. K., Trevisi, E., Tröscher, A., & Vuuren, A. M. van. (2014). Metabolic adaptation during early lactation: Key to cow health, longevity and a sustainable dairy production chain. *CABI Reviews*, *2014*, 1–15. https://doi.org/10.1079/PAVSNNR20149002

74. Kosteli, A., Sugaru, E., Haemmerle, G., Martin, J. F., Lei, J., Zechner, R., & Ferrante, A. W. (2010). Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *The Journal of Clinical Investigation*, *120*(10), 3466–3479. https://doi.org/10.1172/JCI42845

75. Koster, J. D. D., & Opsomer, G. (2013). Insulin Resistance in Dairy Cows. *Veterinary Clinics: Food Animal Practice*, *29*(2), 299–322. https://doi.org/10.1016/j.cvfa.2013.04.002

76. Kovacs, C. S., & Kronenberg, H. M. (1997). Maternal-Fetal Calcium and Bone Metabolism During Pregnancy, Puerperium, and Lactation. *Endocrine Reviews*, *18*(6), 832–872. https://doi.org/10.1210/edrv.18.6.0319

77. Kumar, R., & Thompson, J. R. (2011). The Regulation of Parathyroid Hormone Secretion and Synthesis. *Journal of the American Society of Nephrology : JASN*, *22*(2), 216–224. https://doi.org/10.1681/ASN.2010020186

78. Kumar, V., Abbas, A. K., & Aster, J. C. (2017). *Robbins Basic Pathology: Robbins Basic Pathology E-Book*. Elsevier Health Sciences.

79. LeBlanc, S. (2010). Monitoring Metabolic Health of Dairy Cattle in the Transition Period. *Journal of Reproduction and Development*, *56*(S), S29–S35. https://doi.org/10.1262/jrd.1056S29

80. Lees, P., Landoni, M. F., Giraudel, J., & Toutain, P. L. (2004). Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *Journal of Veterinary Pharmacology and Therapeutics*, *27*(6), 479–490. https://doi.org/10.1111/j.1365-2885.2004.00617.x

81. Lei, M. A. C., & Simões, J. (2021). Invited Review: Ketosis Diagnosis and Monitoring in High-Producing Dairy Cows. *Dairy*, *2*(2), Article 2. https://doi.org/10.3390/dairy2020025

82. Lieben, L., Carmeliet, G., & Masuyama, R. (2011). Calcemic actions of vitamin D: Effects on the intestine, kidney and bone. *Best Practice & Research Clinical Endocrinology & Metabolism*, *25*(4), 561–572. https://doi.org/10.1016/j.beem.2011.05.008

83. Mainau, E., Cuevas, A., Ruiz-de-la-Torre, J. L., Abbeloos, E., & Manteca, X. (2014). Effect of meloxicam administration after calving on milk production, acute phase proteins, and behavior in dairy cows. *Journal of Veterinary Behavior*, *9*(6), 357–363. https://doi.org/10.1016/j.jveb.2014.07.007

84. Majno, G., & Joris, I. (2004). *Cells, Tissues, and Disease: Principles of General Pathology*. Oxford University Press.

85. Malreddy, P. R., Coetzee, J. F., KuKANICH, B., & Gehring, R. (2013). Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *Journal of Veterinary Pharmacology and Therapeutics*, *36*(1), 14–20. https://doi.org/10.1111/j.1365-2885.2012.01384.x

86. Marquardt, J. P., Horst, R. L., & Jorgensen, N. A. (1977). Effect of Parity on Dry Matter Intake at Parturition in Dairy Cattle1. *Journal of Dairy Science*, *60*(6), 929–934. https://doi.org/10.3168/jds.S0022-0302(77)83965-9

87. Martens, H. (2023). Invited Review: Increasing Milk Yield and Negative Energy Balance: A Gordian Knot for Dairy Cows? *Animals*, *13*(19), Article 19. https://doi.org/10.3390/ani13193097

88. Martinez, N., Risco, C. A., Lima, F. S., Bisinotto, R. S., Greco, L. F., Ribeiro, E. S., Maunsell, F., Galvão, K., & Santos, J. E. P. (2012). Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *Journal of Dairy Science*, *95*(12), 7158–7172. https://doi.org/10.3168/jds.2012-5812

89. Martinez, N., Sinedino, L. D. P., Bisinotto, R. S., Daetz, R., Lopera, C., Risco, C. A., Galvão, K. N., Thatcher, W. W., & Santos, J. E. P. (2016). Effects of oral calcium supplementation on mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. *Journal of Dairy Science*, *99*(10), 8397–8416. https://doi.org/10.3168/jds.2015-10527

90. Martinez, N., Sinedino, L. D. P., Bisinotto, R. S., Daetz, R., Risco, C. A., Galvão, K. N., Thatcher, W. W., & Santos, J. E. P. (2016). Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. *Journal of Dairy Science*, *99*(10), 8417–8430. https://doi.org/10.3168/jds.2015-10529

91. Martinez, N., Sinedino, L. D. P., Bisinotto, R. S., Ribeiro, E. S., Gomes, G. C., Lima, F. S., Greco, L. F., Risco, C. A., Galvão, K. N., Taylor-Rodriguez, D., Driver, J. P., Thatcher, W. W., & Santos, J. E. P. (2014). Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. *Journal of Dairy Science*, *97*(2), 874–887. https://doi.org/10.3168/jds.2013-7408

92. Martín-Tereso, J., & Martens, H. (2014). Calcium and Magnesium Physiology and Nutrition in Relation to the Prevention of Milk Fever and Tetany (Dietary Management of Macrominerals in Preventing Disease). *Veterinary Clinics: Food Animal Practice*, *30*(3), 643–670. https://doi.org/10.1016/j.cvfa.2014.07.007

93. Massey, C. D., Wang, C., Donovan, G. A., & Beede, D. K. (1993). Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows. *Journal of the American Veterinary Medical Association*, *203*(6), 852–853.

94. McArt, J. A. A., & Neves, R. C. (2020). Association of transient, persistent, or delayed subclinical hypocalcemia with early lactation disease, removal, and milk yield in Holstein cows. *Journal of Dairy Science*, *103*(1), 690–701. https://doi.org/10.3168/jds.2019-17191

95. McArt, J. A. A., Nydam, D. V., Oetzel, G. R., Overton, T. R., & Ospina, P. A. (2013). Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. *The Veterinary Journal*, *198*(3), 560–570. https://doi.org/10.1016/j.tvjl.2013.08.011

96. McArt, J. A. A., Nydam, D. V., & Overton, M. W. (2015). Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. *Journal of Dairy Science*, *98*(3), 2043–2054. https://doi.org/10.3168/jds.2014-8740

97. McCarthy, M. M., Yasui, T., Felippe, M. J. B., & Overton, T. R. (2016). Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. *Journal of Dairy Science*, *99*(1), 680–700. https://doi.org/10.3168/jds.2015-9694

98. Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, *454*(7203), Article 7203. https://doi.org/10.1038/nature07201

99. Meier, S., Priest, N. V., Burke, C. R., Kay, J. K., McDougall, S., Mitchell, M. D., Walker, C. G., Heiser, A., Loor, J. J., & Roche, J. R. (2014). Treatment with a nonsteroidal antiinflammatory drug after calving did not improve milk production, health, or reproduction parameters in pasture-grazed dairy cows. *Journal of Dairy Science*, *97*(5), 2932–2943. https://doi.org/10.3168/jds.2013-7838

100. Miciletta, M., Cuniberti, B., Barbero, R., & Re, G. (2014). In vitro enantioselective pharmacodynamics of Carprofen and Flunixin-meglumine in feedlot cattle. *Journal of Veterinary Pharmacology and Therapeutics*, *37*(1), 43–52. https://doi.org/10.1111/jvp.12052

101. Miltenburg, C. L., Duffield, T. F., Bienzle, D., Scholtz, E. L., & LeBlanc, S. J. (2016). Randomized clinical trial of a calcium supplement for improvement of health in dairy cows in early lactation. *Journal of Dairy Science*, *99*(8), 6550–6562. https://doi.org/10.3168/jds.2016-10961

102. Mostert, P. F., Bokkers, E. a. M., Middelaar, C. E. van, Hogeveen, H., & Boer, I. J. M. de. (2018). Estimating the economic impact of subclinical ketosis in dairy cattle using a dynamic stochastic simulation model. *Animal*, *12*(1), 145–154. https://doi.org/10.1017/S1751731117001306

103. Mullins, C. R., Mamedova, L. K., Brouk, M. J., Moore, C. E., Green, H. B., Perfield, K. L., Smith, J. F., Harner, J. P., & Bradford, B. J. (2012). Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. *Journal of Dairy Science*, *95*(3), 1323–1336. https://doi.org/10.3168/jds.2011-4744

104. Newby, N. C., Leslie, K. E., Dingwell, H. D. P., Kelton, D. F., Weary, D. M., Neuder, L., Millman, S. T., & Duffield, T. F. (2017). The effects of periparturient administration of flunixin meglumine on the health and production of dairy cattle. *Journal of Dairy Science*, *100*(1), 582–587. https://doi.org/10.3168/jds.2016-11747

105. Newby, N. C., Pearl, D. L., LeBlanc, S. J., Leslie, K. E., von Keyserlingk, M. A. G., & Duffield, T. F. (2013). Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. *Journal of Dairy Science*, *96*(6), 3682–3688. https://doi.org/10.3168/jds.2012-6214

106. Newby, N. C., Renaud, D., Tremblay, R., & Duffield, T. F. (2014). Evaluation of the effects of treating dairy cows with meloxicam at calving on retained fetal membranes risk. *The Canadian Veterinary Journal*, *55*(12), 1196–1199.

107. Newton, K., & Dixit, V. M. (2012). Signaling in Innate Immunity and Inflammation. *Cold Spring Harbor Perspectives in Biology*, *4*(3), a006049. https://doi.org/10.1101/cshperspect.a006049

108. Oetzel, G. R. (1988). Parturient paresis and hypocalcemia in ruminant livestock. *The Veterinary Clinics of North America. Food Animal Practice*, *4*(2), 351–364. https://doi.org/10.1016/s0749-0720(15)31053-7

109. Oetzel, G. R. (1996). Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. *Journal of the American Veterinary Medical Association*, *209*(5), 958–961.

110. Oetzel, G. R. (2015). *Herd-Level Ketosis – Diagnosis and Risk Factors*.

111. Oetzel, G. R., & Miller, B. E. (2012). Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. *Journal of Dairy Science*, *95*(12), 7051–7065. https://doi.org/10.3168/jds.2012-5510

112. Overton, T. R., McArt, J. A. A., & Nydam, D. V. (2017). A 100-Year Review: Metabolic health indicators and management of dairy cattle. *Journal of Dairy Science*, *100*(12), 10398–10417. https://doi.org/10.3168/jds.2017-13054

113. Pascottini, O. B., Leroy, J. L. M. R., & Opsomer, G. (2022). Maladaptation to the transition period and consequences on fertility of dairy cows. *Reproduction in Domestic Animals*, *57*(S4), 21–32. https://doi.org/10.1111/rda.14176

114. Pascottini, O. B., Van Schyndel, S. J., Spricigo, J. F. W., Carvalho, M. R., Mion, B., Ribeiro, E. S., & LeBlanc, S. J. (2020). Effect of anti-inflammatory treatment on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-62103-x

115. Qu, Y., Fadden, A. N., Traber, M. G., & Bobe, G. (2014). Potential risk indicators of retained placenta and other diseases in multiparous cows. *Journal of Dairy Science*, *97*(7), 4151–4165. https://doi.org/10.3168/jds.2013-7154

116. Quiroz-Rocha, G. F., LeBlanc, S. J., Duffield, T. F., Wood, D., Leslie, K. E., & Jacobs, R. M. (2009). Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. *The Canadian Veterinary Journal*, *50*(4), 383–388.

117. Radostits, O. M., Blood, D. C., & Gay, C. C. (1994). Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses. *Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses., Ed. 8*. https://www.cabdirect.org/cabdirect/abstract/19942214281

118. Rajala-Schultz, P. J., Gröhn, Y. T., & McCulloch, C. E. (1999). Effects of Milk Fever, Ketosis, and Lameness on Milk Yield in Dairy Cows. *Journal of Dairy Science*, *82*(2), 288–294. https://doi.org/10.3168/jds.S0022-0302(99)75235-5

119. Rastani, R. R., Grummer, R. R., Bertics, S. J., Gümen, A., Wiltbank, M. C., Mashek, D. G., & Schwab, M. C. (2005). Reducing Dry Period Length to Simplify Feeding Transition Cows: Milk Production, Energy Balance, and Metabolic Profiles. *Journal of Dairy Science*, *88*(3), 1004–1014. https://doi.org/10.3168/jds.S0022-0302(05)72768-5

120. Reinhardt, T. A., Lippolis, J. D., McCluskey, B. J., Goff, J. P., & Horst, R. L. (2011). Prevalence of subclinical hypocalcemia in dairy herds. *The Veterinary Journal*, *188*(1), 122–124. https://doi.org/10.1016/j.tvjl.2010.03.025

121. Rhoads, M. L., Rhoads, R. P., VanBaale, M. J., Collier, R. J., Sanders, S. R., Weber, W. J., Crooker, B. A., & Baumgard, L. H. (2009). Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin1. *Journal of Dairy Science*, *92*(5), 1986–1997. https://doi.org/10.3168/jds.2008-1641

122. Risco, C. A., Drost, M., Thatcher, W. W., Savio, J., & Thatcher, M. J. (1994). Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postrartum dairy cows. *Theriogenology*, *42*(1), 183–203. https://doi.org/10.1016/0093-691X(94)90675-9

123. Roche, J. R., Kay, J. K., Friggens, N. C., Loor, J. J., & Berry, D. P. (2013). Assessing and Managing Body Condition Score for the Prevention of Metabolic Disease in Dairy Cows. *Veterinary Clinics: Food Animal Practice*, *29*(2), 323–336. https://doi.org/10.1016/j.cvfa.2013.03.003

124. Rodríguez, A. R., Palma, P. I., Solar, M. A., & Bustamante, H. A. (2021). Early postpartum treatment with carprofen in a dairy herd with high incidence of clinical metritis – a case study. *Journal of Applied Animal Research*, *49*(1), 139–146. https://doi.org/10.1080/09712119.2021.1909033

125. Rodríguez, E. M., Arís, A., & Bach, A. (2017). Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. *Journal of Dairy Science*, *100*(9), 7427–7434. https://doi.org/10.3168/jds.2016-12210

126. Schmitt, R., Pieper, L., Borchardt, S., Swinkels, J. M., Gelfert, C.-C., & Staufenbiel, R. (2023a). Effects of a single transdermal administration of flunixin meglumine in early postpartum Holstein Friesian dairy cows: Part 1. Inflammatory and metabolic markers, uterine health, and indicators of pain. *Journal of Dairy Science*, *106*(1), 624–640. https://doi.org/10.3168/jds.2021-20555

127. Schmitt, R., Pieper, L., Borchardt, S., Swinkels, J. M., Gelfert, C.-C., & Staufenbiel, R. (2023b). Effects of a single transdermal administration of flunixin meglumine in early postpartum Holstein Friesian dairy cows: Part 2. Milk yield, culling risk, and reproductive performance. *Journal of Dairy Science*, *106*(1), 641–652. https://doi.org/10.3168/jds.2022-21995

128. Seely, C. R., Wilbur, C. N., Fang, K. M., & McArt, J. A. A. (2024). Effects of timing of oral calcium administration on milk production in high-producing early-lactation Holstein cows. *Journal of Dairy Science*, *107*(3), 1620–1629. https://doi.org/10.3168/jds.2023-23541

129. Seifi, H. A., LeBlanc, S. J., Leslie, K. E., & Duffield, T. F. (2011). Metabolic predictors of post-partum disease and culling risk in dairy cattle. *The Veterinary Journal*, *188*(2), 216–220. https://doi.org/10.1016/j.tvjl.2010.04.007

130. Sheldon, I. M., Cronin, J. G., & Bromfield, J. J. (2019). Tolerance and Innate Immunity Shape the Development of Postpartum Uterine Disease and the Impact of Endometritis in Dairy Cattle. *Annual Review of Animal Biosciences*, *7*(Volume 7, 2019), 361–384. https://doi.org/10.1146/annurev-animal-020518-115227

131. Shock, D. A., Renaud, D. L., Roche, S. M., Poliquin, R., Thomson, R., & Olson, M. E. (2018). Evaluating the impact of meloxicam oral suspension administered at parturition on subsequent production, health, and culling in dairy cows: A randomized clinical field trial. *PLOS ONE*, *13*(12), e0209236. https://doi.org/10.1371/journal.pone.0209236

132. Shwartz, G., Hill, K. L., VanBaale, M. J., & Baumgard, L. H. (2009). Effects of flunixin meglumine on pyrexia and bioenergetic variables in postparturient dairy cows1. *Journal of Dairy Science*, *92*(5), 1963–1970. https://doi.org/10.3168/jds.2008-1581

133. Sordillo, L. M., Contreras, G. A., & Aitken, S. L. (2009). Metabolic factors affecting the inflammatory response of periparturient dairy cows. *Animal Health Research Reviews*, *10*(1), 53–63. https://doi.org/10.1017/S1466252309990016

134. Stilwell, G., Schubert, H., & Broom, D. M. (2014). Short communication: Effects of analgesic use postcalving on cow welfare and production. *Journal of Dairy Science*, *97*(2), 888–891. https://doi.org/10.3168/jds.2013-7100

135. Suthar, V. S., Canelas-Raposo, J., Deniz, A., & Heuwieser, W. (2013). Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *Journal of Dairy Science*, *96*(5), 2925–2938. https://doi.org/10.3168/jds.2012-6035

136. Swartz, T. H., Schramm, H. H., Bewley, J. M., Wood, C. M., Leslie, K. E., & Petersson-Wolfe, C. S. (2018). Meloxicam administration either prior to or after parturition: Effects on behavior, health, and production in dairy cows. *Journal of Dairy Science*, *101*(11), 10151–10167. https://doi.org/10.3168/jds.2018-14657

137. Trevisi, E., Amadori, M., Cogrossi, S., Razzuoli, E., & Bertoni, G. (2012). Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. *Research in Veterinary Science*, *93*(2), 695–704. https://doi.org/10.1016/j.rvsc.2011.11.008

138. Trevisi, E., & Minuti, A. (2018). Assessment of the innate immune response in the periparturient cow. *Research in Veterinary Science*, *116*, 47–54. https://doi.org/10.1016/j.rvsc.2017.12.001

139. Trimboli, F., Ragusa, M., Piras, C., Lopreiato, V., & Britti, D. (2020). Outcomes from Experimental Testing of Nonsteroidal Anti-Inflammatory Drug (NSAID) Administration during the Transition Period of Dairy Cows. *Animals*, *10*(10), Article 10. https://doi.org/10.3390/ani10101832

140. Tveit, B., Lingaas, F., Svendsen, M., & Sjaastad, Ø. V. (1992). Etiology of Acetonemia in Norwegian Cattle. 1. Effect of Ketogenic Silage, Season, Energy Level, and Genetic Factors. *Journal of Dairy Science*, *75*(9), 2421–2432. https://doi.org/10.3168/jds.S0022-0302(92)78003-5

141. Valldecabres, A., Pires, J. A. A., & Silva-del-Río, N. (2018). Effect of prophylactic oral calcium supplementation on postpartum mineral status and markers of energy balance of multiparous Jersey cows. *Journal of Dairy Science*, *101*(5), 4460–4472. https://doi.org/10.3168/jds.2017-12917

142. van Knegsel, A. T. M., Remmelink, G. J., Jorjong, S., Fievez, V., & Kemp, B. (2014). Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. *Journal of Dairy Science*, *97*(3), 1499–1512. https://doi.org/10.3168/jds.2013-7391

143. Venjakob, P. L., Borchardt, S., & Heuwieser, W. (2017). Hypocalcemia—Cow-level prevalence and preventive strategies in German dairy herds. *Journal of Dairy Science*, *100*(11), 9258–9266. https://doi.org/10.3168/jds.2016-12494

144. Venjakob, P. L., Pieper, L., Heuwieser, W., & Borchardt, S. (2018). Association of postpartum hypocalcemia with early-lactation milk yield, reproductive performance, and culling in dairy cows. *Journal of Dairy Science*, *101*(10), 9396–9405. https://doi.org/10.3168/jds.2017-14202

145. Venjakob, P. L., Staufenbiel, R., Heuwieser, W., & Borchardt, S. (2021). Association between serum calcium dynamics around parturition and common postpartum diseases in dairy cows. *Journal of Dairy Science*, *104*(2), 2243–2253. https://doi.org/10.3168/jds.2019-17821

146. West, J. W. (2003). Effects of Heat-Stress on Production in Dairy Cattle. *Journal of Dairy Science*, *86*(6), 2131–2144. https://doi.org/10.3168/jds.S0022-0302(03)73803-X

147. White, H. M. (2015). The Role of TCA Cycle Anaplerosis in Ketosis and Fatty Liver in Periparturient Dairy Cows. *Animals*, *5*(3), Article 3. https://doi.org/10.3390/ani5030384

148. Wilhelm, A. L., Maquivar, M. G., Bas, S., Brick, T. A., Weiss, W. P., Bothe, H., Velez, J. S., & Schuenemann, G. M. (2017). Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. *Journal of Dairy Science*, *100*(4), 3059–3067. https://doi.org/10.3168/jds.2016-11743

149. Wilkens, M. R., Nelson, C. D., Hernandez, L. L., & McArt, J. A. A. (2020). Symposium review: Transition cow calcium homeostasis—Health effects of hypocalcemia and strategies for prevention. *Journal of Dairy Science*, *103*(3), 2909–2927. https://doi.org/10.3168/jds.2019-17268

150. Wysolmerski, J. J. (2012). Osteocytic osteolysis: Time for a second look? *BoneKEy Reports*, *1*, 229. https://doi.org/10.1038/bonekey.2012.229

151. Zhang, F., Nan, X., Wang, H., Zhao, Y., Guo, Y., & Xiong, B. (2020). Effects of Propylene Glycol on Negative Energy Balance of Postpartum Dairy Cows. *Animals*, *10*(9), Article 9. https://doi.org/10.3390/ani10091526

152. Zhang, G., & Ametaj, B. N. (2020). Ketosis an Old Story Under a New Approach. *Dairy*, *1*(1), Article 1. https://doi.org/10.3390/dairy1010005

153. Waelchli, R.O., R. Thun, and H. Stocker. 1999. Effect of flunixin meglumine on placental expulsion in dairy cattle after a caesarean. Veterinary Record 144:702–703. doi:10.1136/vr.144.25.702